Population Biology and Restoration of Intertidal Cockle Beds.

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Abstract

There is evidence that infaunal bivalves in New Zealand are not as abundant as they once were with overfishing and habitat modification contributing to the decline in density and health of cockles. The population biology and abundance of the bivalve *Austrovenus stutchburyi* (tuangi) in eight beds in four estuaries was assessed both seasonally (13 seasons) and annually (7 years) as little is known about the cockle beds in the Canterbury region of New Zealand. As with populations of similar species worldwide, there were site specific differences in population structure (density and size ranges) with the highest densities at Takamatua (>1500/m^2), and the lowest at Port Levy (<350/m^2). Gonad indices varied between male and female cockles. Male reproductive cycles were similar at all sites with male cockles being reproductively active year round, while females were more active in spring and summer. Temporal and spatial site specific differences occurred in cockle condition with high salinity sites having higher condition indices (CI) than low salinity sites. There were spatial and temporal variations in salinity (3-35ppt), sediment structure (fine sand through to predominantly silt), water temperature (6-20°C), nutrient supply (total volatile solids (TVS) 0.002- 0.15mg/L) and contaminant levels. Metal pollution indices (MPI) ranged between 3 and 11.

Three cockle transplant trials were undertaken both within and between estuarine systems. Caged cockles survived well, and cage design needed to allow vertical movement of the bivalves within the substrate to reduce mortality. At the end of the 12 month trial, approximately 45% of the cockles remained in the plots. The condition of transplanted cockles was similar to naturally occurring cockles. Recommendations are made to optimise the success of cockle transplants. Large scale, un-caged placement of 25-30mm length cockles in the mid-low tide region of areas with stable, but not necessarily uncontaminated substrate, moderate salinity and temperature and with a reliable nutrient supply is recommended. The results from the thesis research can be applied to other infaunal bivalves in New Zealand allowing more successful restoration processes leading to increased species diversity and ecosystem functioning.

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For Jim.

Ehara taku toa i te toa takitahi engari he toa takitini

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Chapter 1

General Introduction

Background

While bivalves have traditionally been a food resource for maritime communities, they are also important components of the ecosystems in which they live, giving shape and stability to the substrates in which they burrow, and distributing particles by way of the process of filtration. Over the last decade, shellfish bed depletions have been recorded from many parts of the world (Airoldi & Beck 2007; Polyakov et al. 2007; Genelt-Yanovskiy et al. 2010; Vassiliev et al. 2010; Watanabe & Katayama 2010; Yan et al. 2010; Ravit et al. 2012), in both natural and commercial beds. Research has shown that as well as human exploitation (Atkinson et al. 2010), environmental factors such as temperature, salinity and food supply (Tallis et al. 2004; Hofman et al. 2006; Leontarakis et al. 2008; Guerra et al. 2012) and sediment quality and dynamics (Bouma et al. 2001; Huxham & Richards 2003) play a role in population structure and sustainability with excessive siltation reducing feeding efficiency. Grain size preferences also influence growth rates, abundance and survival (Hermann et al. 2009; Lundquist et al. 2009) with coarse sediments inhibiting burrowing and often leading to shell damage (Global invasive species database, 2007).

Larval recruitment and development are a major component in population dynamics of bivalves including *Mercenaria mercenaria* (Przeslawski & Webb 2009) with the number of recruits being related to poor larval growth rates and survival. A number of studies on *Cerastoderma edule* (Flach 1996), and *Macoma balthica* (Richards et al. 2002; Huxham & Richards 2003; Strasser et al. 2003; Bos 2006) suggest that larval recruitment success was related to lower adult stock density. Dethier (2010), who found that sites with high recruitment did not necessarily have large adult populations, postulates that predation on juveniles, adult-juvenile interactions and physiologically stressful abiotic conditions play important roles in community structure.

Population declines and compromised shellfish health can be related to human induced activities including habitat modification (Johnson & Heck 2007), industrial contaminants, urban development, land run off and increased sedimentation (Healey 1980, De Luca-Abbott et al. 2000, Stewart 2005, Norkko et al. 2006, Hewitt & Norkko 2007; Heggie & Savage 2009; Thelen & Thiet 2009). Herrman et al. (2009) found that a density decrease in *Donax hanleyanus* (wedge clam) was related to human trampling especially on beaches where tourism was a major activity. Eutrophication of coastal waters with associated hypoxia has led to a decrease in commercial production of *Ruditapes philippinarum* (Manila clam) in Japan (Watanabe & Katayama 2010) while research in the Arctic has shown that a reduction in sea ice cover and thickness could lead to a shift in food references from phytoplankton to epibenthic algae with associated community structure changes (Sun et al. 2009). On the other hand, reduced tidal flow over clam beds has led to decreased early survivorship and growth of *Mercenaria*

mercenaria (Powers 2009), and can lead to reduced salinity with obvious changes to estuarine community structure and function (Thelen & Thiet 2009).

Pre- and post-settlement processes are important structuring agents of invertebrate populations and communities (Olafsson et al. 1994; Cummings et al. 1995; Todd 1998; Menge 2000; Gosling 2003). Predation is documented as having large impacts on dispersing larvae (Andre & Rosenberg, 1991; Hunt & Scheibling, 1997; Huxham & Richards 2003) and along with competition, physical disturbance and physiological stress will lead to increased mortality of newly-settled larvae (Todd, 1998). Also, bivalve densities may decline through predation impacts on larval recruits (Beukema & Dekker 2005) which can be related to a warming climate where the increased abundance of predators such as shrimps is climate related.

Shellfish are part of the natural history of New Zealand, a small country with an extensive coastline. In a country with no natural mammals, these were an important food source along with birds for the first settlers arriving here. Even today, the gathering of kai moana (sea food) is an important activity for many people. However, overuse of these resources has led to restrictions on the number of individuals gathered and on how they are gathered.

There is strong evidence that in New Zealand infaunal bivalves are not as abundant as they once were (Cole et al. 2000; Grant & Hay, 2003) and it has been established that recruitment for the New Zealand cockle *Austrovenus stutchburyi* (a Venerid clam) is extremely variable (Larcombe, 1971; Marsden & Knox, 2008; Kainamu 2010) with many sites showing spatial and temporal variations as seen in the northern hemisphere (Dare et al. 2004; Beukema & Dekker 2005).

Commercial cockle beds are monitored on a regular basis having been introduced to the Quota Management Scheme (QMS) in October 2002. The Total Allowable Commercial Catch (TACC) was set at 3214 metric tonnes for the 20011/12 fishing year (Seafood Industry Council), the same level as preceding years. The accepted minimum size for commercial harvesting is 30mm in length. For this same period the annual catch allowance for customary and recreational fishing was set at 161000 and 221000 kgs respectively. Recreational fishing is limited to 150 cockles per person per day outside reserve areas, with no size restriction, with the total take in the COC 3B region (of which Canterbury is a part) being 55000kg (1000kg commercial; 27000kg each for customary and recreational catch).

Using the intertidal marine bivalve *Austrovenus stutchburyi* as a model, this thesis research investigated factors that may have resulted in declines in shellfish beds, and methods and techniques of restoration. Endemic throughout New Zealand (Morton & Miller 1973) Austrovenus *stutchburyi is* an important species to recreational fishers, has cultural significance and is of economic value, with commercial fisheries for cockles having existed around Whangarei Harbour since the 1970s, at Nelson (Golden Bay, 1984) and in Otago (1986). As well as having an impact on commercial harvesting, declining cockle numbers have an ecological effect through the loss of a food source for wading birds such as oystercatchers, which have been estimated to account for 20% of the annual consumption of cockles in the Avon-Heathcote estuary (Healey 1980; Baker 1969).

Little is known about cockle populations in the Canterbury Westland area other than the population in the Avon-Heathcote/Ihutai estuary (Stephenson 1981; Pilkington 1992; Marsden & Pilkington 1995). Mussel aquaculture is increasing in Pegasus Bay and Banks Peninsula with potential impacts on the sustainability of adjacent cockle beds. There is a world-wide deficit in the knowledge of the effects of aquaculture on nearshore communities. North Island cockle beds are known to be depleted through overharvesting and disease (Stewart *&* Creese 2002). Although cockle populations in the Canterbury area are highly variable, similar declines have been recorded on Banks Peninsula. Current data from a Port Levy/Koukourārata (Banks Peninsula) bed (Voller 2006) closed to harvesting for a 7 year period shows low, stable densities, a situation similar to Auckland beds closed for a comparable length of time (Morrison & Brown 1999).

Bivalve growth is affected by salinity and nutrient supply (Iglesias et al. 1992: Gosling 2003; Marsden 2004; Mouritsen 2002 ;) as well as by sediment loading and contamination of the water (Navarro 1988; Fegley et al. 1992; Stewart & Creese 2002). Pilkington (1992) found that cockle condition was correlated to chlorophyll α concentration and salinity at low tide, with two sites in the Avon-Heathcote/Ihutai estuary showing appreciable differences in cockle condition. Cockles from the bed close to the estuary outlet had greater growth and reproductive output than those from within the estuary where salinity fluctuations were greater. Research (Remane 1934: Mann et al 2005; Anibal et al 2011) has shown that this is a worldwide phenomenon with European cockles also showing growth retardation relative to low salinity levels.

Austrovenus stutchburyi is a filter feeding estuarine bivalve inhabiting the area from below low tide to mean high tide levels (Stephenson 1981; Morton & Miller 1973).

Feeding occurs when the animal is submerged, with the shell valves opening to allow the short siphons to extend. The feeding activity is regulated by endogenous circatidal rhythms of valve movement (Beentjes & Williams, 1986). Filter-feeding bivalves can accumulate contaminants, although the effect of this varies between species (Absil et al. 1996).

Cockle condition is a measure of the growth, reproduction and survival of populations as well as individuals. Environmental factors that may affect condition include competition, sedimentation, parasitism, pollution, salinity, hypoxia, temperature, and nutrient availability. Cockle condition is the culmination of the interaction of many factors and gives a measure on the "health" or physiological state of the animal (Pilkington 1992) with a reduction in the condition being associated with physiological stress (Pridmore et al 1992) and so a reduction in the metabolic processes. The bivalve condition index can be a measure of a variety of relationships: wet flesh weight to wet shell weight; dry flesh weight to dry shell weight; glycogen to dry flesh weight; glycogen to protein ratio; dry tissue weight to shell cavity volume (Pilkington 1992). These can all show seasonal variations, and anomalies can occur when epibionant effects are not recognised. Epibionts, organisms which attach to the outside of the shell valves, may have positive (Mouritsen & Poulin 2003) or negative (Norkko 2005) effects on cockle condition measurements. Measurement of nuclei acid ratios, where changes at the molecular level can indicate changes occurring at tissue levels, can be used to monitor short-term growth rates with higher values being related to faster growth (Norkko 2005). A combination of these two assessment methods may give a more precise indication of the growth and reproductive potential of shellfish.

Environmental stressors such as contaminants, eutrophication and nutrient loading along with competition and parasitism, temperature, salinity levels and hypoxia all impact on cockle health, and consequently the reproductive and recruitment potential. Competition from other species for suspended nutrients, and parasite infestation both lead to nutrient and energy reduction which will affect the growth and reproduction of the cockles.

Sedimentation alters the ability of bivalves to extract phytoplankton from the water and so may change nutrient availability (Iglesias et al. 1992; Navarro 1988). Contaminants can also be introduced in association with these particles. Increased sediments and contaminant loadings may also be linked to eutrophication which may have deleterious effects, although Shriver et al. (2002) determined that land–derived N loads did not alter the condition of bay scallops and the additional nutrients may have resulted in higher growth rates. Sedimentation can also affect current flows which can lead to a reduction in seston concentrations in the near bottom layers (Marsden 2004; Dolmer 1999).

Increasing water temperatures can impact on reproductive output as energy is diverted from gonad development to general metabolic processes to cope with the warmer environment. Warmer water also has a lower oxygen-carrying capacity. Any reduced oxygen availability associated with sedimentation will result in reduced gas exchange efficiency which in turn will impact on the general metabolism of the animal. Energy demands can also be increased through pre-ingestive selection processes where the organic quality and particle size of filtered matter results in increased pseudofaeces production (Gosling 2003).

Cockle densities may decline through predation impacts on larval recruits (Beukema & Dekker 2005) which can be related to a warming climate, as well as sediment changes. Cockle mortality has been shown to increase through parasitism with infestation causing the bivalves to come to the substrate surface while feeding (Desclaux et al. 2002). Parasite infection may contribute to increased uptake of contaminants. Research with northern hemisphere species showed that cockles infected with a digenean trematode had increased concentrations of contaminants in their tissues (Baudrimont et al. 2005).

Biological interactions also affect bivalves, and cockle mortality has been shown to increase through parasitism (Perrigault et al. 2010) with some infestations causing the bivalves to come to the substrate surface while feeding (Poulin et al. 2000; Desclaux, et al. 2002).

It has been documented that the New Zealand cockle *Austrovenus stutchburyi* is prone to trematode infestation of the foot (Mouritsen 2002) which reduces the burrowing ability of the animals, leaving them vulnerable to predation. Also, a relationship has been established between parasite infestation and cockle condition (Mouritsen & Poulin 2003), with poor condition being linked with greater parasite numbers.

Habitat restoration seeks to return an ecosystem back to an original (historical) state. In reality this is not always feasible and rehabilitation or habitat improvement may be a more realistic goal. Sustainability can be described as the continuity of a system. In this case it applies to the maintenance of the environment of the shellfish beds with the view to allowing them to remain productive areas. While natural recruitment may lead to the recovery of cockle beds, it appears that harvesting bans alone may not be effective in the short to medium term as recruitment of *Austrovenus stutchburyi* is known to be sporadic (Stewart 2005) and extremely variable (Larcombe 1971) and bed enhancement may be necessary. Enhancement or rejuvenation of an environment can be achieved by modifying the existing habitat to make better use of natural and artificial larval recruitment, and also by introducing mature individuals to boost natural larval recruitment (Morrison & Browne 1999; Cummings et al. 2007).

Research to date on *Austrovenus stutchburyi* shows that transplantation is possible using adult cockles (Cummings et al. 2007) with lesser success being achieved using juveniles. Preliminary research in Whangateau Harbour (Stewart 2002) using manipulative field experiments looked at factors affecting growth and survival of transplanted individuals, and also investigated the seasonal timing for successful transplantation. Further research in this region continues on the effects of urbanisation on cockle populations (Stewart 2005; Thrush et al 2003). Factors that impact in this region close to Auckland city may be quite different to those that affect shellfish populations on South Island east coast beaches. In North America successful enhancement of estuarine bivalve populations have been achieved through the out-planting of hatchery-produced spat (Morrison & Browne 1999) but whether this could be an option in New Zealand needs further investigation.

Aims and Objectives

Cockle populations from eight sites around Banks Peninsula and the Canterbury region (see Figure 2.1) were investigated to establish their population structure and condition (Pilkington, 1992) with particular regard to the effects of salinity and sediment contamination. It has been documented that *Austrovenus stutchburyi* can tolerate prolonged low salinity exposure as long as nutrient quality and quantity are adequate, otherwise become stressed and lose condition and weight (Marsden 2004). This is similar to the response shown by the northern hemisphere bivalves *Mercenaria mercenaria* (Arnold et al. 1991) and *Macoma balthica* (Harvey & Vincent 1991).

Environmental factors including salinity, temperature and nutrient availability were assessed for each of the sites and sediment analysis was performed to determine the degree of site contamination and trace metal levels. The purpose was to relate population statistics to sediment, food availability and water quality. Histological techniques were used to investigate reproductive condition (Marsden 2004), and parasite infestation, as these can be related to bivalve condition which in turn can be linked to the success of a population.

The research examined the effects of parasitism, pollution and human impacts on cockle beds with and without a history of anthropogenic inputs. Field experiments determined the optimum size for transfer and the reproductive success of transplanted bivalves. The procedures and protocols developed are able to be applied to other bivalve species with a view to maintaining and managing native shellfish stocks.

By relating population characteristics, condition and reproductive potential of cockles to sediment and water quality conditions this research assessed how environmental conditions affect the health, growth and reproduction of shellfish. It examined the effects of parasitism, pollution, and human impacts along with environmental stressors such as eutrophication, nutrient loading and contaminant.

The potential for habitat restoration or improvement to enhance shellfish bed productivity was also investigated. Shellfish bed enhancement by seeding techniques assessed the growth and reproductive success of transplanted mature individuals over time.

The key questions being addressed in this research are;

1. Does the population structure vary between cockle beds in the Canterbury region?
2. Do environmental conditions affect the health, growth and reproduction of shellfish? I.e. does cockle condition correlate with environmental factors, in particular salinity, sediment and contaminants?
3. Are cockle beds in the Canterbury region in decline?
4. Can cockle beds be successfully enhanced by habitat restoration or improvement?
5. Can cockle transplants be used to improve or maintain the long term sustainability of shellfish beds?

Continuing on from Chapter 1, the study areas of Saltwater Creek, Avon-Heathcote Estuary, Port Levy/Koukourārata and Akaroa Harbour and the sampling methods are presented in Chapter 2. The general procedures for population monitoring, and monitoring of environmental variables are also described, as is the place of *Austrovenus stutchburyi* in the estuarine community.

Population abundances, and size distribution of *Austrovenus stutchburyi* were surveyed seasonally for 13 consecutive seasons (Jan 2006 – Jan 2009) and for 7 consecutive summers (Jan 2006-Jan 2012) and these results are presented in Chapter 3, along with the results of the analysis of the environmental variables, salinity, sediment grain size, sediment trace metals and nutrients, temperature and water quality (TVS). Differences in population structure were tested for correlation with these environmental variables.

Chapter 4 describes seasonal cycles in reproduction and cockle condition. Glycogen content and the size of the cockles were tested for correlations with condition indices. Histological assessment of gonad material allowed the correlation of the reproductive state with the size and glycogen content of cockles from all the sampling areas. The relationships between growth and reproduction with environmental factors were also investigated.

Chapter 5 describes three transplant experiments which varied both in size (the number of bivalves transplanted) and timeframe (weeks to years) with the view of establishing guidelines for the monitoring, management and potential restoration of shellfish beds. Survival and condition indices were used to compare the physiological health of transplanted and natural cockles in relation to environmental variables.

The results of the preceding chapters are brought together in Chapter 6 and the key questions addressed. A set of protocols for cockle transplants are also presented.

Chapter 2

Study areas and general methodology

Introduction

The Canterbury region lies on the flood plains of two large braided river systems, the Waimakariri to the north, and the Rakaia to the south, both of which are fed from the Southern Alps lying to the west. To the east is the Pacific Ocean and adjacent to the coalescing fans of the plains, the Port Hills, ancient extinct volcanoes which make up Banks Peninsula. Flowing through the area are several smaller rivers, including the Ashley, the Styx, the Avon and the Heathcote (Figure 2.1).

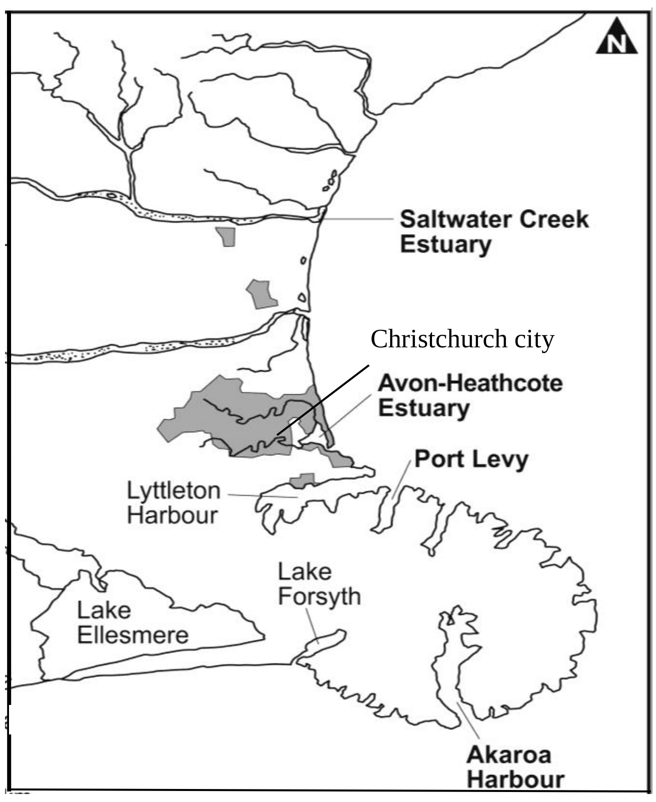


Figure 2.1: Map of the Canterbury region showing the main rivers and streams. Study sites in bold font.

Study Sites

The sites chosen for the study (Table 2.1) vary in catchment area, surrounding topography, sediment structure, exposure, and urbanisation and are located in four areas, all of which can be classified as estuaries (Hume et al, 2007). Within each of the four area, two sites were selected, one with high salinity, the other with low.

Table 2.1: Global positioning reference coordinates for the 8 Canterbury sites. Shaded cells identify low salinity sites.

Area	Site	Coordinates (GPS)
Saltwater Creek	Saltwater Creek A	S 43° 16.056’ E 172° 43.024’
	Saltwater Creek B	S43° 16.135’ E 172° 43.292’
Avon-Heathcote Estuary	Bromley	S43° 32.488’ E172° 43.279’
	Tern St	S43°33.232’ E172° 44.535’
Port Levy	Fernlea	S 43° 39.637’ E 172° 49.093’
	Pa	S43°39.171’ E 172° 49.868’
Akaroa Harbour	Takamatua	S43° 46.893’ E172° 57.805”
	Barry’s Bay	S43° 45.752’ E172° 54.875’

Saltwater Creek Estuary

Lying to the north of Christchurch city, Saltwater Creek estuary (Figure 2.2) is the focus of a variety of both active and passive activities including bird-watching (this area is an important bird refuge supporting a variety of wading and migratory seabirds), fishing, white-baiting and duck-shooting. This estuary is the meeting point of the Ashley

River, which arises in the foothills and Saltwater Creek, a lowland waterway, whose water channels connect to form a shallow lagoon.

The catchments of the two waterways are both modified by human activity, particularly pastoral agriculture with its attendant runoff of both natural (faecal matter) and artificial fertilisers. Timber processing was carried out in the immediate catchment in the past (Andrew McKenzie; farmer, pers. comm.) and the area was a port during early European settlement times.

Figure 2.2: Aerial view of Saltwater Creek Estuary showing study sites.

According to Hume et al. (2007) the hydrodynamic processes in this estuary are dominated by river flows, with the main channel being relatively well-flushed. The large sand bank separating this lagoon from the ocean affords some protection from easterly winds.

Figure 2.3: Views of the Saltwater Creek estuary at (A) low tide and (B) high tide.

Avon-Heathcote/Ihutai Estuary

The Avon-Heathcote/Ihutai Estuary (Figure 2.4), lying to the east of Christchurch City, and also the confluence of two waterways, is surrounded by urban development and protected from the ocean by a sand spit. It has a narrow mouth, a shallow basin with complex shorelines and the large intertidal area is bisected with drainage channels. The hydrodynamic processes are tide driven (Hume et al. 2007; Hart et al. 2008). Salinity levels are close to those in the sea towards the mouth of the estuary but are lower at the head of the estuary and at the river mouths (Pilkington 1992; Hume et al. 2007). As well as supporting large and diverse bird populations, this estuary is also a recreational area with water-sports and fishing.

Initial development of Christchurch city involved drainage of the natural wetlands with a big input of fine loess material into both rivers (Harris 1992). Until 1925 this silt was trapped by introduced watercress and willows. After this time remedial work addressing the problem of congested rivers lead to the silt finding its way into the estuary. By the late 1950’s the silt influx had reduced, eelgrass was re-establishing and the expansion of Bromley sewage treatment plant ended the direct discharge of septic tanks into the estuary. The rivers were no longer used to dispose of industrial and urban waste, with the subsequent build-up of heavy metals and other pollutants associated with industry.

When this research commenced (summer 2006), water from the Bromley sewage treatment plant was discharged on the out-going tide from the landward side of the estuary. In March 2010 a pipeline running under the estuary was commissioned allowing the waste water to be discharged 7kms offshore into Pegasus Bay. Since the earthquakes of September 2010, February 2011 and June 2011, the waste water treatment process has been compromised with some untreated material being discharged into the estuary and the pipeline is not operational. The sediments in the Bromley region are prone to being anoxic. Also the earthquakes have affected the drainage patterns of the estuary, as the land has risen by approximately 40cm at the seaward end and the mouth of the Heathcote and dropped by a similar amount at the mouth of the Avon.

Figure 2.4: Aerial view of the Avon-Heathcote/Ihutai estuary showing study sites.

Figure 2.5: Views of the Tern St (Avon-Heathcote/Ihutai Estuary) site at (A) low tide and (B) high tide.

Figure 2.6: The Bromley site at low tide.

The other 4 sites are located on Banks Peninsula, a peninsula of volcanic origin on the east coast of the South Island of New Zealand. It has an area of approximately 1,150 square kilometres (440 sqm) and encompasses two large harbours and many smaller bays and coves.

Port Levy/Koukourārata

Port Levy is an elongated inlet with a wide entrance adjacent to and south of Lyttleton harbour and with extensive mudflats at the head of the harbour. The wide entrance allows the water circulation to be ocean forced (Hume et al, 2007; Hart et al. 2008) and is the site of commercial mussel farming operations (Figure 2.7)

Figure 2.7: Mussel farm at the entrance to Port Levy/Koukourārata inlet.

Figure 2.8: Aerial views of the Fernlea (A) and Pa (B) sites, Port Levy/Koukourārata.

No harvesting of cockles had been allowed since 1995 due to consistently low cockle density (Voller 2006) at the Pa site adjacent to the Koukourārata marae on the south side of the harbour. The cockles found at this site are generally larger than cockles at the other sites, and surveys carried out by the Rūnunga since 1997 show the bed as stable but not increasing in density (Voller 2006). The other site, alongside the discharge point of Fernlea Stream is at the head of the harbour. This harbour is surrounded by steeply sloping hills which are used as farmland.

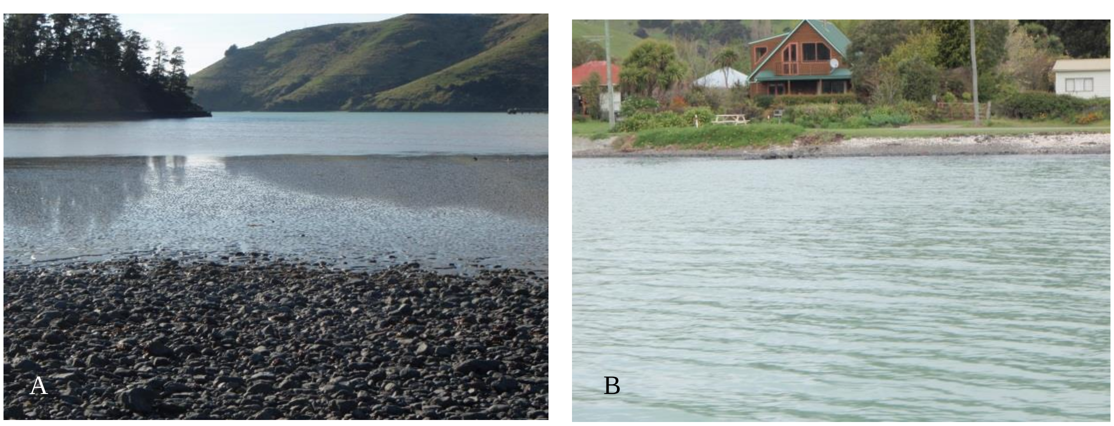


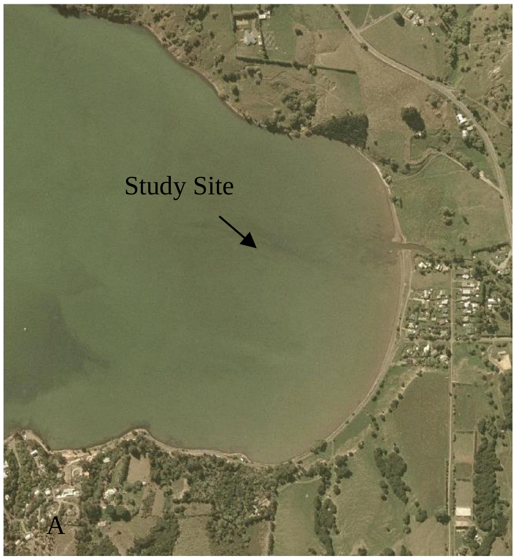
Figure 2.9: The Pa site at (A) low and (B) high tide.

Figure 2.10: Views of the Fernlea site at (A) low and (B) high tide.

Akaroa Harbour

Akaroa Harbour has a narrow entrance to the sea, and the hydrodynamics are ocean driven as the volume of river flow during a tidal cycle is small in comparison to the total estuary volume (Hume et al 2007; Hart et al. 2008). The sites were selected for substrate as well as salinity differences. Takamatua has approximately 23% of its substrate as silt/clay and is exposed to wind-generated waves. Barry's Bay, at the head of the harbour is made up of 95% silt/clay (Bolton-Ritchie 2005) and is protected from the south by the Onawe Peninsula, the site of a once fortified Ngāi Tahu Pā and is characterized by large areas of mudflats indicative of low exposure to wind-driven waves.

Takamatua (rest after journey) was a stopover point for Māori as they moved around the Peninsula. With European settlement the surrounding land was used for dairy and sheep farming and in recent times there has been an increase in housing subdivision with episodes of sediment run-off (Boulton-Ritchie pers. comm.2008).



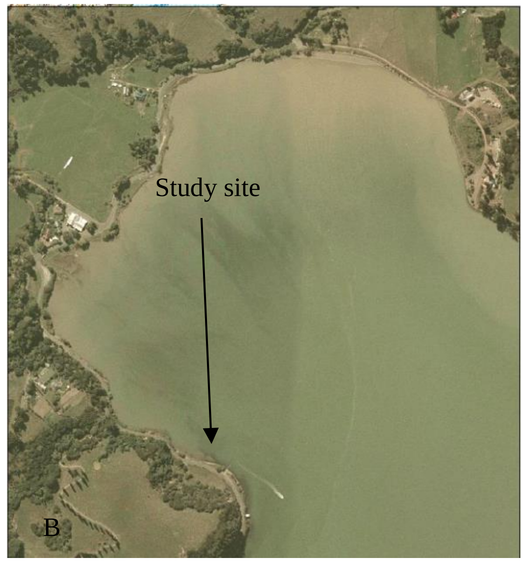


Figure 2.11: Aerial views of the (A) Takamatua and (B) Barry's Bay sites.

Figure 2.12: Looking across the mudflats at Barry's Bay

An preliminary survey of the intertidal fauna over the summer of 2006 at all sites showed similar patterns of species diversity (Table 2.2) with cockles being the dominant species followed by the mud-flat snail and assorted polychaetes.

Table 2.2: Species list from the 8 Canterbury sites in the low-tide region.

Site / Species	Common name	SWA	SWB	Bromley	Tern St	Fernlea	Pa	Takamatua	Barry's Bay
Algae									
Gracilaria spp.					x				**x**
Ulva spp.	Sea lettuce			x	x				
Enteromorpha spp				x	x				
Zostera novazelandica	Sea grass			x	x				
Worms									
Unidentified polychaetes		x	x	x	x	x	x	x	x
Unidentified oligochaete		x	x			x			
Mollusca									
Amaurochiton glaucus	Green chiton		x						
Amphibola crenata	Mudflat snail	x	x	x	x	x		x	x
Turbo smaragdus	Cats eye					x	x		x
Micrelenchus tenebrosus	Topshell			x	x	x	x		x
Diloma subrostrata	Mudflat topshell			x	x	x			
Cominella glandiformis	Mudflat whelk			x	x	x		x	x
Cominella maculosa	Spotted whelk			x	x	x			
Elminius modestus	Estuarine barnacle		x	x	x	x			**x**
Macomona liliana	Wedge shell	x	x	x	x	x	x	x	x
Austrovenus stutchburyi	NZ cockle	x	x	x	x	x	x	x	**x**
Paphies australis	Pipi	x	x				x		
Paphies donacina	Tuatua	x	x			x		x	x
Tiostrea chilensis	Rock oyster					x	x		x
Xenostobus pulex	Little black mussel			x	x	x			

Crustacea									
Macrophthalmus hiripes	Stalk-eyed mud crab				x		x	x	x
Helice crassa	Tunneling mud crab	x	x	x	x	x		x	x
Ovalipes catharus	Paddle crab				x				
Anthozoa									
Edwardsia leucomelos	Anemone			x					
Anthopleura aureoradiata	Anemone			x	x				
Fishes									
Rhombosolea plebeia	Sand flounder, dab			x	x				
Kathetostoma giganteum	Star gazer			x					

Austrovenus stutchburyi

Endemic throughout New Zealand (Morton & Miller, 1973) *Austrovenus stutchburyi* is a dominant member of the soft shore community where it's presence has a stabilizing effect on the substrate. Known also as the New Zealand cockle or tuangi, *A.stutchburyi* belongs to the family Veneridae and can be viewed as the ecological equivalent of *Mercenaria mercenaria* and *Cardium edule* found in the northern hemisphere (Pilkington 1992). This sessile, burrowing bivalve occurs at depths of 2-4cm generally from the mid-tide to the below the low tide level. It is a filter feeder, with gut contents corresponding to that of the overlying water (Stephenson 1981). Feeding takes place when the animal is submerged by the extension of siphons fringed with tentacles to prevent the entry of sand grains (Levington 1991). Growth rates are generally about 10mm per year for the first two years, slowing after this to reach 30mm in length by age four. It is accepted that *A.stutchburyi* becomes sexually mature at about 18mm in length irrespective of age (Stephenson 1981). *A.stutchburyi* is classed as a long-lived species. Pilkington (1992) found estuarine cockles (subjected to regular tidal cycles of exposure) aged up to 9 years, and marine ones (limited exposure at neap tides) up to 12 years, while Stephenson (1981) suggested an age in excess of 20 years.

An important species to recreational fishers, *A.stutchburyi* has cultural significance and is of economic value, with commercial fisheries for cockles having existed around Whangarei Harbour since the 1970's, at Nelson (Golden Bay) and in Otago. As well as having an impact on commercial harvesting, declining cockle numbers impact ecologically through the loss of a food source for crabs, birds and fish. They also have an important role in benthic-pelagic coupling aiding the transfer of energy from the pelagic to the benthos (Kainamu 2010). In addition, they contribute to the physical stability of the substrate and can provide attachment surfaces for epibionts.

Figure 2.13: A New Zealand cockle moving through the substrate at low tide at Saltwater Creek.

General Methodology

Population surveys

At each site, a minimum of three quadrats were taken along a transect/s running from the low to mid-tide level at each of the four seasons over the first three years (Jan 2006-Jan 2009) always in the same months for each season. Annual sampling was continued (December-January) for the following four years (2009-2012). Sediment was removed from within each of the 250 x 250mm quadrats to a depth of 100mm and sieved through a 1mm mesh sieve. Shell length of 100 animals was measured in the field to the nearest mm and the cockles returned to their original site. Sub-samples (10 cockles) were collected for condition analysis (Norkko 2005) and histological examination of gonad development and parasite infestation.

Sediment content and particle size

Winter collected 10cm deep x 10cm diameter cores from each site were analysed for particle size using a Saturn Digisizer 5200™. The samples were oven dried at 35°C, sieved through a 2mm sieve and underwent nitric/hydrochloric acid digestion prior to analysis to determine the percentage composition in the following divisions: <3.9; 3.9-7.7; 7.8-14.99; 15-31; 32-62; 63-124; 125-249; 250-499; 500-999; 1000+ microns.

Sediment nutrients and trace metals

Three replicate core samples for analysis of sediment nutrients and trace metals were collected from each site during winter 2006 and summer 2006/2007. 5cm deep x 5cm diameter cores were collected into pre-cleaned plastic containers supplied by Hill Laboratories Hamilton, NZ, an IANZ an credited laboratory and chilled prior to sending to the laboratory. Samples were analysed for the nutrients nitrogen (N) and phosphorus (P) and seven contaminants, arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb) and zinc (Zn).

Physical variables

Intertidal length, the region between the low tide and tide levels was measured for each site. Surrounding land use and human impacts were assessed by identifying what the land was used for (urban- rural), the topography, (steepness of adjacent land), human impacts (eg fishing, recreational activities) and the impact of other species (e.g. bird predation) for each of the eight sites. These were ranked on a scale of one to eight, 1 being lowest, 8 highest impact. Temperature was recorded over a 12 month period (April 2007-April 2008) using Hoboware© data loggers positioned just above the level of the substrate (Figure 2.13) set to record at 2 minute intervals. Water temperatures at high tide and air temperature at low tide were extracted from these data. Salinity was measured at each sampling period using a hand held Atago S-10 salinity refractometer.

Figure 2.14: Data logger attached to marker post.

Experimental, biochemical & histological techniques

Procedures for analysing condition index, gonad index and glycogen levels are described in the relevant chapters, as are the designs and procedures of the cockle transplant trials.

Data analysis

Data were analysed on different scales to show site-specific and between site temporal variability. All univariate analyses were performed using STATISTICA©6. Differences in density, cockle size, condition, and reproductive state were tested using general linear models. Spearman Rank correlation analysis (p=0.5) was used to test the relationship between population structure, condition, glycogen levels, and the environmental variables (sediment, temperature, salinity nutrients, trace metals, and land use.

Data are presented graphically for each of the paired sites in the 4 areas. The physical characteristics of the sites were tabulated.

Chapter 3

Population structure

Introduction

Cockles are a group of the many species of shellfish collected worldwide by recreational and commercial gatherers. Due to changes in environmental conditions and increased pressure from exploitation over the last decade, shellfish beds have been depleted (Airoldi & Beck 2007; Polyakov et al 2007; Marsden & Adkins 2010; Genelt-Yanovskiy et al. 2010; Vassiliev et al. 2010; Watanabe & Katayama 2010; Yan et al. 2010). Estuarine shellfish experience a wide range of environmental conditions including salinity, temperature variations, food availability and changing sediment quality (Bouma et al. 2001; Huxham & Richards 2003; Hofman et al.2006; Jayawickrema & Wijeyaratne 2009; Atkinson et al. 2010). Pre- and post-settlement processes are also important structuring agents for invertebrate populations and communities (Olafsson et al. 1994; Cummings et al. 1995; Todd 1998; Menge 2000; Mann et al. 2005). All these factors are assumed to play a role in the on-going sustainability of shellfish beds, but few studies have identified the interacting effects of environmental conditions on the population density and population structure of bivalves.

The population dynamics for any given organism can be related to the habitat in which it occurs (Krebs, 2001), including the resources available and the physical environment. To make valid comparisons between populations, these parameters must also be considered. The purpose of this research was to characterise the 8 study sites, including sediment structure and quality, water and air temperature, salinity, intertidal length (which can influence the exposure time of sediment-dwelling organisms) and any anthropogenic influences arising from the use of the adjacent land areas.

Salinity levels have a major effect on physiological processes of estuarine bivalves. Exposure to lower salinity results in mussels and clams reducing oxygen uptake and filtration rates which reduces their scope for growth (Navarro 1988; Marsden 2004) and may lead to increased mortality (Widdows 1985, Shriver et al. 2002). If bivalves have reduced growth rates when exposed to low salinity conditions then this may be reflected in the population structure and the maximum shell length (Remane 1934; Tallis 2004). Human induced activities such as eutrophication, habitat modification (Johnson & Heck 2007), industrial contaminants, urban development, land run off and increased sedimentation (De Luca-Abbott et al. 2000, Stewart 2005, Norkko et al. 2006, Hewitt & Norkko 2007; Heggie & Savage 2009; Thelen & Thiet 2009, Watanabe & Katayama 2010) have been linked to population declines and compromised shellfish health. To understand the effects of the environment, especially environmental changes, on population structure it is necessary to follow populations annually. Seasonal monitoring is important to follow recruitment and mortality patterns (Hewitt & Thrush 2007; Hewitt & Thrush 2009a; 2009b) but to date in Canterbury this has not been done.

Larval recruitment and development are a major component in population dynamics (Przeslawski & Webb 2009) with the number of recruits being related to poor larval growth rates and survival. A number of studies (Flach 1996; Richards et al. 2002; Huxham & Richards 2003; Strasser et al. 2003; Bos 2006) suggest that larval recruitment success was related to lower adult stock density. Dethier (2010), who found that sites with high recruitment did not necessarily have large adult populations, postulates that predation on juveniles, adult-juvenile interactions and physiologically stressful abiotic conditions play important roles in community structure. Larval dispersal also plays a part in pre-settlement site recruitment processes, with some juveniles (up to 2mm in length) exhibiting secondary dispersal through drifting in the tidal currents (Ramón 2003).

It has been demonstrated that environmental factors such as temperature, salinity and food supply (Hofman et al.2006; Anibal et al. 2011; Yang et al. 2011) and sediment quality and dynamics (Bouma et al. 2001; Huxham & Richards 2003) play a role in population structure and sustainability with excessive siltation reducing feeding efficiency. Grain size preferences also influence growth rates, abundance and survival (Hermann et al. 2009; Lundquist et al. 2009) with coarse sediments inhibiting burrowing and often leading to shell damage (Global invasive species database, 2007).

The filter feeding bivalve *Austrovenus stutchburyi*, (Finlay 1927), is an important species to recreational fishers. Endemic throughout New Zealand (Morton & Miller 1973), it has cultural significance and is of economic value, with commercial fisheries for cockles having existed around Whangarei Harbour since the 1970s, at Nelson (Golden Bay) and in Otago. There is evidence that infaunal bivalves in New Zealand are not as abundant as they once were (Cole et al. 2000; Grant & Hay 2003) and research indicates that recruitment of *Austrovenus stutchburyi* is extremely variable (Larcombe 1971; Marsden & Knox, 2008) with many sites showing spatial and temporal variations as seen in similar bivalve species in the northern hemisphere (Dare et al. 2004; Beukema & Dekker 2005).

Since their inclusion in the Quota Management Scheme (QMS) in October 2002, commercial cockle beds are monitored on a regular basis, with the Total Allowable Commercial Catch (TACC) being set at 3214 metric tonnes for the 20010/11 fishing year (Seafood Industry Council). The accepted minimum size for commercial harvesting is 30mm shell length. Recreational fishing is limited to 150 cockles per person per day outside reserve areas, with no size restriction. While records are regularly kept on the population size and structure of cockles from commercial cockle beds there is less information on beds from South Island sites apart from early and unpublished research from the Avon-Heathcote/Ihutai Estuary (Stephenson 1981; Pilkington 1992; Marsden & Pilkington,1995).

Mussel aquaculture is increasing in Pegasus Bay and Banks Peninsula with potential impacts on the sustainability of adjacent cockle beds. Many studies of this bivalve species in the Canterbury region have been one off surveys or short term experiments and little is known of the seasonal and annual changes in population size and structure. Seasonal and long term studies including recruitment information are needed to establish whether populations of this relatively long lived species are likely to survive environmental perturbations.

The aim of this study was to determine the variability in population structure and density for *Austrovenus stutchburyi* in the mid- to low-tide level (Stephenson, 1981; Morton & Miller 1973) in four estuarine systems along the Canterbury coast. In particular the effects of salinity and sediment contaminants on the population parameters were to be evaluated.

It was predicted that the populations would be variable due to differences in environmental parameters, aspect and human impacts on the areas. This is the first in-depth evaluation of cockle beds in the South Island and at the time of writing the largest record in terms of timeframe and number of sites monitored for this region. As such, the results will provide a useful baseline for monitoring in other parts of New Zealand. The information can also be used in conjunction with research on North Island cockle beds (Stewart 2005; Cummings et al 2007; Lundquist et al 2009) to assess which environmental parameters are important in evaluating suitable restoration sites for this species in New Zealand. The data collected also provides a valuable comparison of the state of these beds pre-Canterbury earthquakes (4^{th} September 2010) and the impact of natural disruption such as liquefaction in the Avon-Heathcote/Ihutai Estuary.

Methods

Population surveys

Commencing in January 2006, populations of *Austrovenus stutchburyi* were measured for density and size frequency at each of two sites within the 4 survey areas (Saltwater Creek Estuary, Avon-Heathcote/Ihutai Estuary, Port Levy/Koukourārata and Akaroa Harbour) giving a total of eight sites (Figure 2.1, Chapter 2). Site selection was based on differing salinity levels, with sediment structure and intertidal lengths being similar within areas. Use of the surrounding land (Marsden et al. 2008) varied as did the fishing pressure on the various sites. Surveys were conducted each spring (September-November), summer (December-February), autumn (March–May) and winter (June-

August) until January 2009, a total of 13 surveys. Annual surveys were continued during January 2010, 2011 and 2012 giving a total of 7 summer surveys. Summer surveys were chosen for the extended study for two reasons. The surveys originally began with a summer survey, so this was the way of optimising the data set. The second consideration was being able to monitor recruitment from spring/early summer spawning.

Bivalves (100) from 3 or more random quadrats (25 x 25 cm) were measured in the field to the nearest mm in length and returned to their original site (see Chapter 2). Sub-samples (10) were collected for condition analysis and histological examination of gonad development and parasite infestation (see Chapter 4). Salinity of the water from a nearby channel was measured at low tide at each survey.

Sediment analysis

Particle size

Sediment samples were collected as described in the General methodology section of Chapter 2.

Nutrients and Trace metals

Analysis of sediment nutrients (N and P) and trace metals (As, Cd ,Cr, Cu, Ni, Pb and Zn) from samples collected during the winter and early summer of 2006 were determined at Hill Laboratories, Hamilton, NZ, an IANZ accredited laboratory. Total metal content of the seven metals at the different sites was compared using the metal pollution index (MPI) calculated using the equation

$$MPI= (Cf_1 \times Cf_2 \ldots\ldots Cf_n)^{1/n}$$

where Cf_n is the concentration of the metal n in the sample (Usero et al. 2004).

Temperature, Salinity & Land Use

Temperature was recorded over a 12 month period using Hoboware© data loggers (see Chapter 2, Figure 2.13) set to record at 2 minute intervals. Water temperatures at high tide and air temperature at low tide were extracted from these data.

Salinity was measured at each site at each sampling time using a hand-held Atago S-10 salinity refractometer. Intertidal length(m) was determined for each site, as well as a ranked (1(lowest) - 8 (best)) assessment of surrounding land use based on the degree of urbanisation of surrounding areas, the amount and type of agriculture and other uses to which the areas were put.

Microorganics

Triplicate 2 litre water samples were collected seasonally at high tide at each of the eight sites. In the laboratory, one litre subsamples were filtered through 0.45µm pre-weighed Millipore filters, then dried at 60°C for 72 hours, weighed, and then ashed at 450°C for 5 hours. The residue was weighed and the percentage of total volatile solids (TVS) per litre were calculated for each sample using the formula:

TVS =(filtrate pre ashed weight- filtrate ashed weight)/filtrate pre ashed weight.

Data analysis

Data were analysed on different scales to investigate site-specific and between site temporal variability. All univariate analyses were performed using STATISTICA© 6. Differences in density and cockle size over time were tested using general linear models. Data were tested for normality and transformed where necessary to meet those assumptions. The relationship between population structure and the environmental variables (sediment, temperature, salinity nutrients, trace elements, and land use impacts was tested using Spearman Rank correlation analysis (p=0.5).

Results

Density

The relative abundance of *Austrovenus stutchburyi* based on mean densities determined seasonally and annually varied both between sites and seasons (Figure 3.1), and between years within sites (Figure 3.2). There was a significant difference in cockle density between high and low salinity sites over the 13 seasons (Table 3.1, p <0.001) and the 7 summers (Table 3.2).

At Saltwater Creek both the high and low salinity sites were similar initially with densities of less than 500 cockle/m^2 (Figure 3.1A). Site A (lower salinity) showed a marked increase to more than 1500/m^2 over the summer of 2009, with this gain dropping away over the following 12 months to around 750 cockles/m^2, and remaining so through to the 2012 survey. Site B (higher salinity) showed increased densities at both the summer 2007 and 2010 surveys to approximately 800 cockles/m^2 (Figure 3.2A).

In the Avon-Heathcote/Ihutui Estuary (Figure 3.1B) the lower salinity Bromley site consistently had densities of 200-300 cockles/m^2 while at the Tern Street site (higher salinity) densities often reached 1500-2000 cockles/m^2. The summer surveys of 2010 (pre-earthquake), 2011 and 2012 found lower densities at both these sites (Figure 3.2B) with Tern Street having densities between 500-1000 cockles/m^2 and Bromley less than 200.

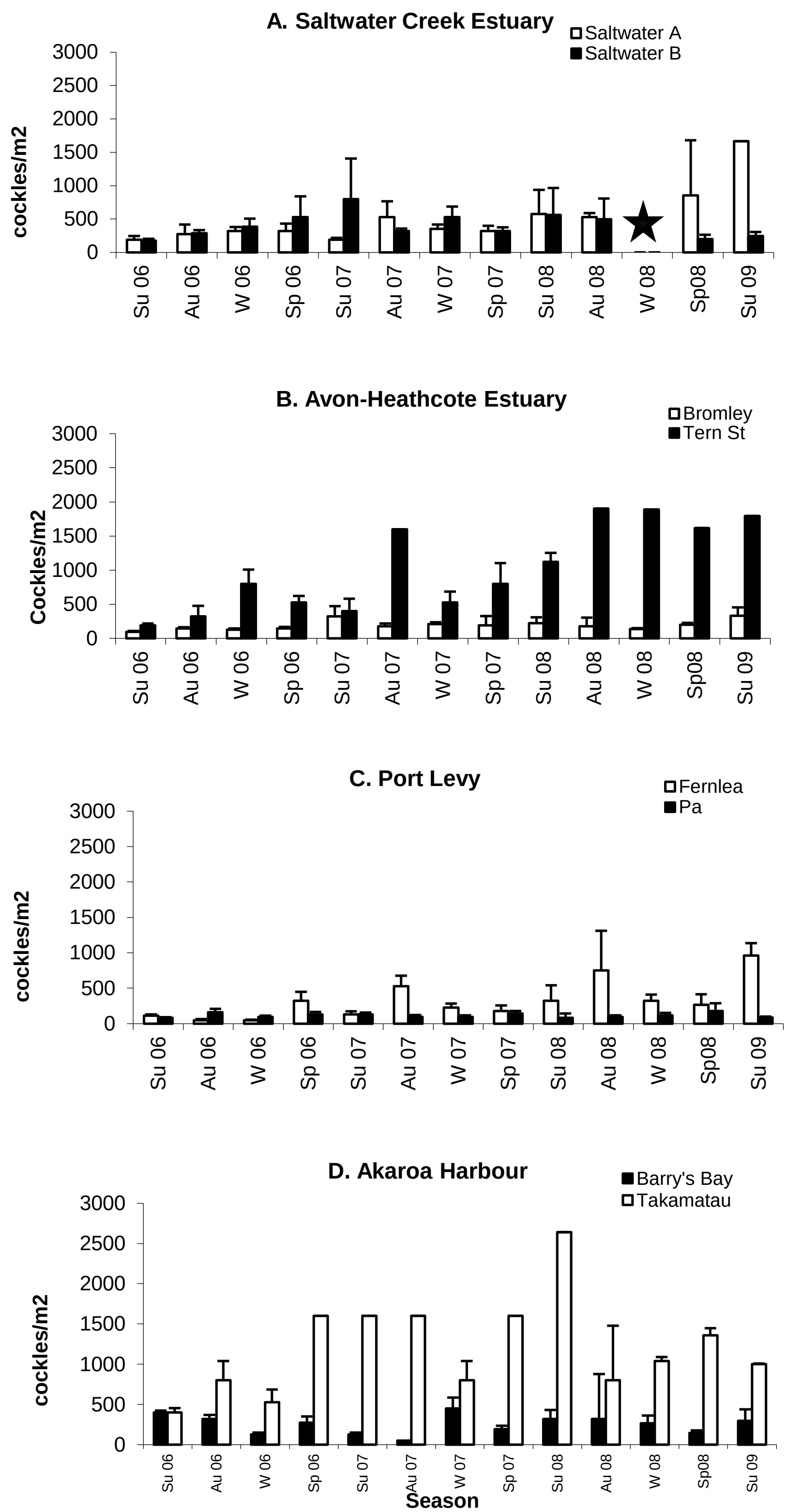


Figure 3.1: Effect of season on population density for the 8 Canterbury sites (mean +/- 1SE) for 13 seasonal surveys (summer 06- summer 09). Dark bars identify higher salinity sites. ★ No sample.

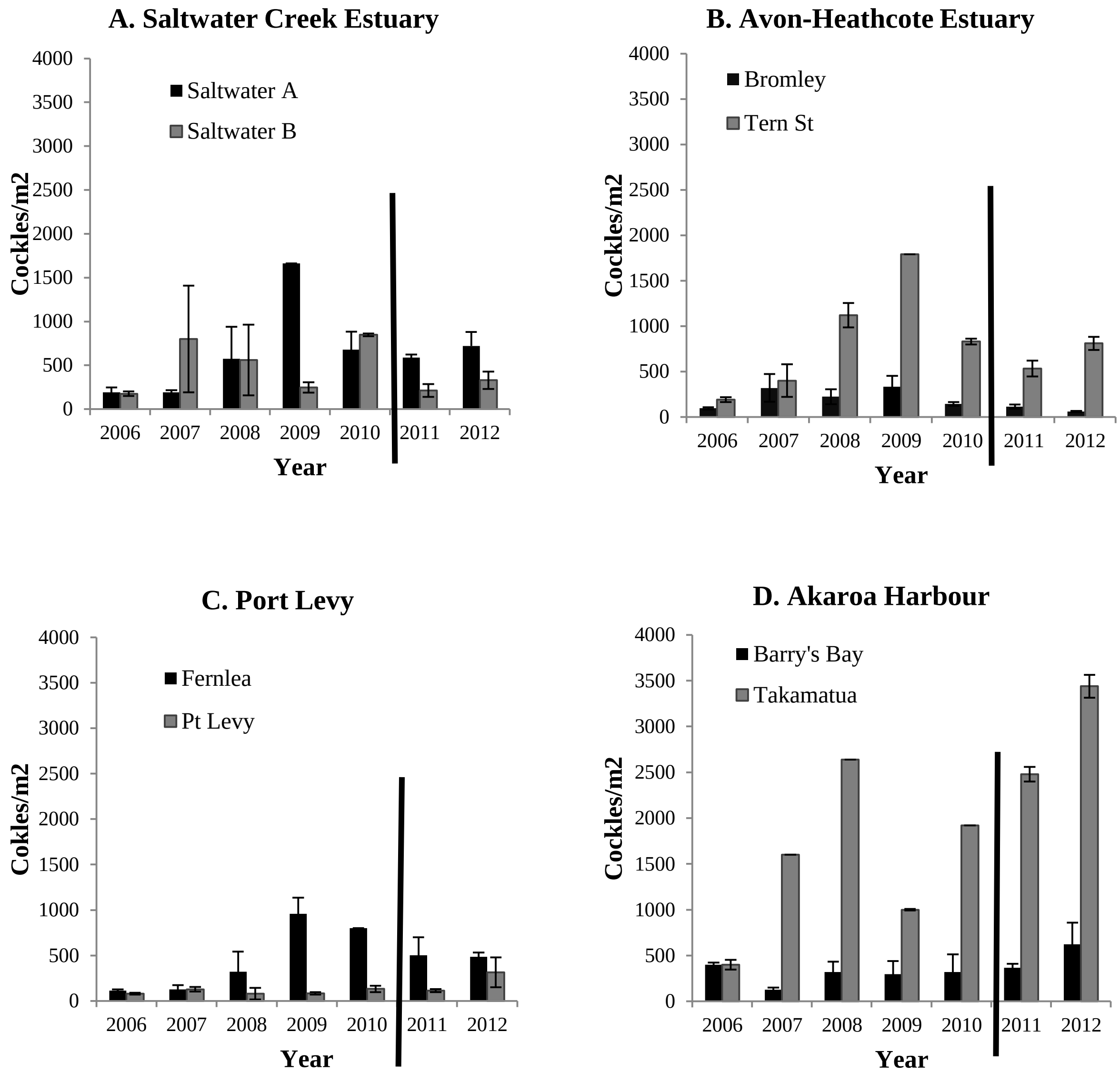


Figure 3.2: Annual variation in the density of *Austrovenus stutchburyi* in summer (2006-2012) comparing low and high salinity site in 4 estuaries (mean +/- 1SE). Dark bars identify higher salinity sites. Vertical lines denote September 4th 2010 earthquake.

Table 3.1: Results of single factor ANOVA comparing mean seasonal density of *Austrovenus stutchburyi* at high and low salinity sites. Significant values are shown in bold.

Source	***df***	***MS***	***F value***	***P-value***
Salinity	1	18206	22.24	**>0.001**
Area	24	818.8		

Table 3.2: Results of 2 way ANOVA comparing density of *Austrovenus stutchburyi* at high and low salinity sites at each of the 4 estuaries over 13 seasons. Significant values are shown in bold.

Area	*df*	*MS*	*F value*	*P-value*
Saltwater Creek	1	228.12	0.58	0.45
Avon-Heathcote	1	18206	22.24	**<0.001**
Port Levy	1	1125	7.58	0.111
Akaroa Harbour	1	246.5	32.01	**<0.001**

Table 3.3: Results of 2 way ANOVA comparing density of *Austrovenus stutchburyi* at high and low salinity sites at each of the 4 estuaries over 7 summers (2006-2012). Significant values are shown in bold.

Area	*df*	*MS*	*F value*	*P-value*
Saltwater Creek	1	147087.5	0.909	0.36
Avon-Heathcote	1	1374322	9.38	**0.009**
Port Levy	1	402562.6	7.33	**0.0019**
Akaroa Harbour	1	8677463	15.94	**0.0018**

The Port Levy/Koukourārta sites (Fig 3.1C) both had low population densities for all seasons, although Fernlea (lower salinity) had higher numbers (500-1000 cockles/m^2) occurring at summer/autumn surveys suggesting recruitment inputs. The summer survey of 2012 showed a marked increase in cockle density at the higher salinity Pa site to 300 cockles/m^2 (Figure 3.2 C).

At the lower salinity Takamatua site, cockle density was high, often greater than 1500 cockles /m^2 and over 3000 cockles/m^2 in 2012 (Figure 3.2D), but size classes were small (Figure 3.3). Barry's Bay, the higher salinity Akaroa Harbour site (Fig. 3.1D) consistently had densities of less than 500 cockles/m^2.

Size class range

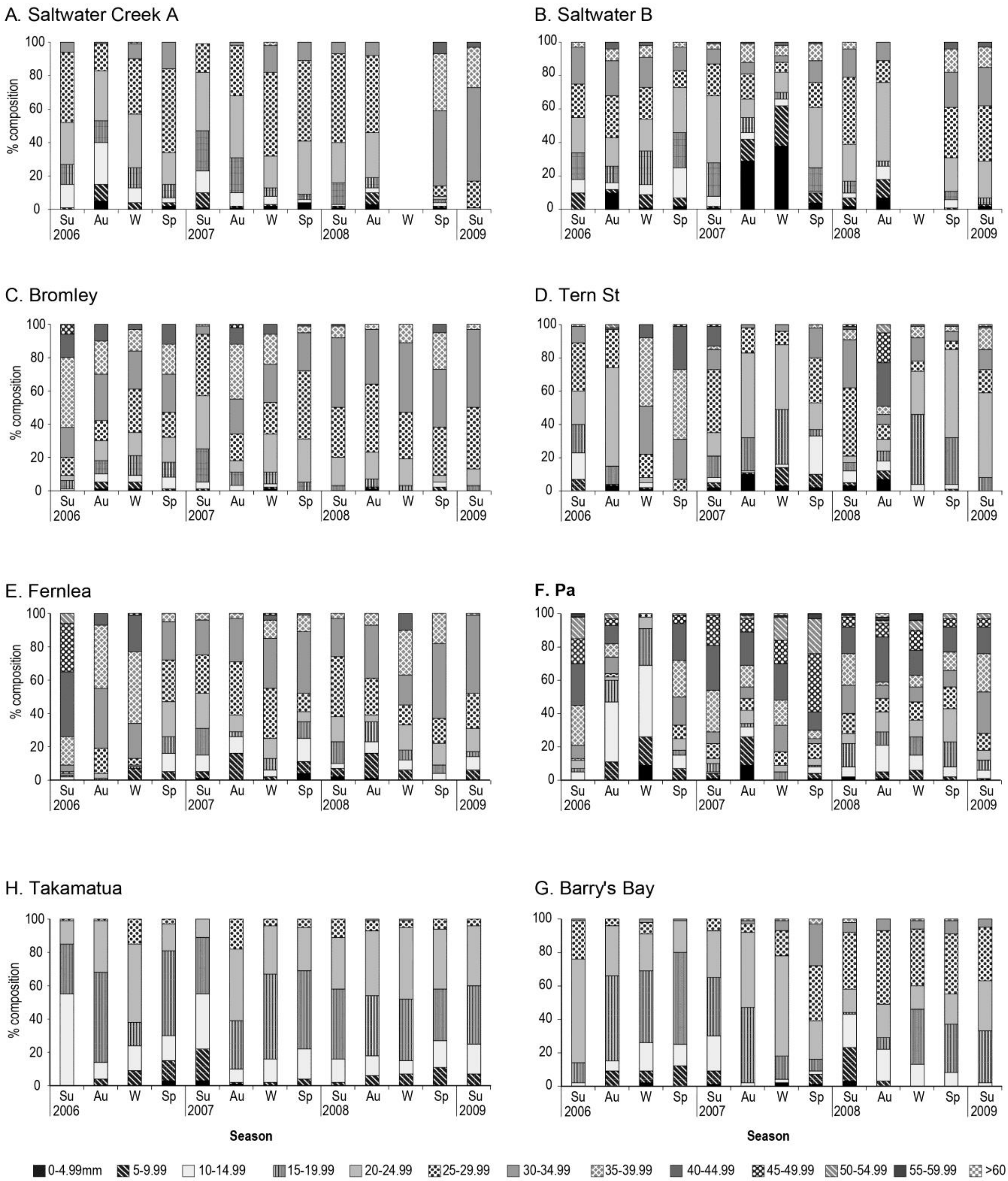


Figure 3.3: Population class divisions based on shell length for the 8 Canterbury sites for the 13 seasonal surveys from Dec 2006 to Jan 2009.

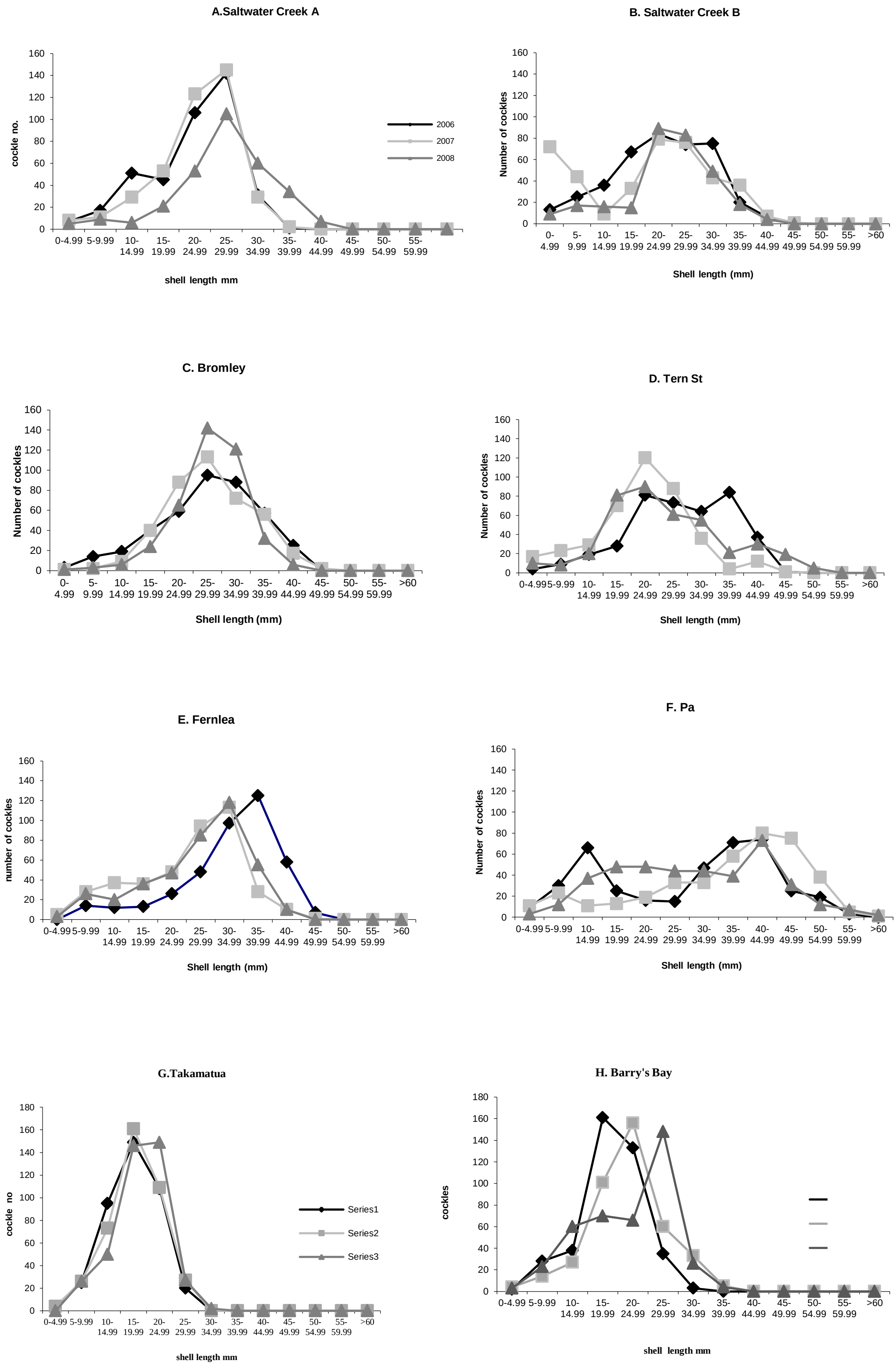


Figure 3.4: Annual mean shell length size classes for the 8 Canterbury sites for years 2006, 2007, & 2008.

The mean shell length at all sites showed both seasonal and site differences (Figures 3.3 & 3.4), with the low salinity Takamatua site consistently having smaller length cockles and the high salinity Pa site having the largest cockles. There was no significant difference in the mean cockle size between high and low salinity sites (Table 3.4, *p*= 0.058). The sites exhibited a uni-modal population structure (Figure 3.4) except for the Pa site at Port Levy/ Koukourārata .

Table 3.4: 2-way ANOVA results comparing mean seasonal cockle length between high and low salinity sites. Significant values are shown in bold.

Source	*df*	*MS*	*F value*	*p-value*
Site	1	107.43	3.97	0.058
Season	24	27.04		
Total	25			

The size of cockles present at each site is presented as size frequency histograms for seasonal data (Figure 3.3A-H) and as linear graphs to illustrate annual (summer) (Figure 3.4A-H) population structure.

At Saltwater Creek, site A, lower salinity, in the upper estuary (Fig 3.3A), had a population consisting predominantly of adult individuals. Site B, higher salinity, closer to the estuary mouth (Fig 3.4B) also had a definite adult population but showed a peak of small (<5mm length) individuals in 2007 suggesting recruitment.

In the Avon-Heathcote/Ihutai Estuary, the Bromley site (lower salinity) (Fig 3.3C) had lower recruitment inputs than at the higher salinity Tern Street site (Fig 3.3D). The timing of the recruitment differed between the 2 sites, with Bromley showing a small influx over autumn/winter of 2006. Two peaks showed at Tern St; one between autumn and spring of 2007 and the second during the autumn of 2008.

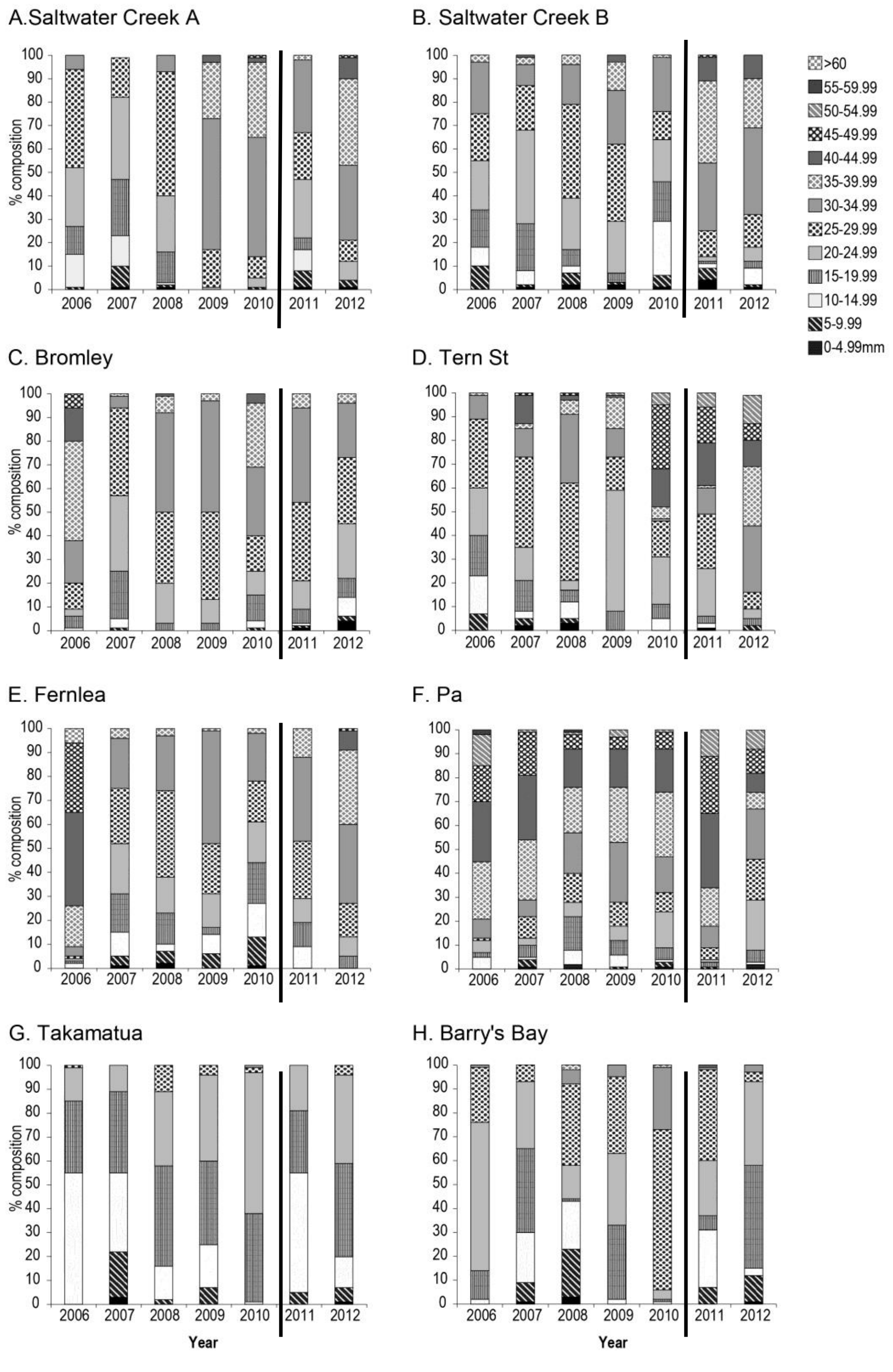


Figure 3.5: Population class divisions based on shell length for the 8 Canterbury sites for the 7 summer surveys for years 2006 to 2012. Vertical bars denote September 4th 2010 earthquake.

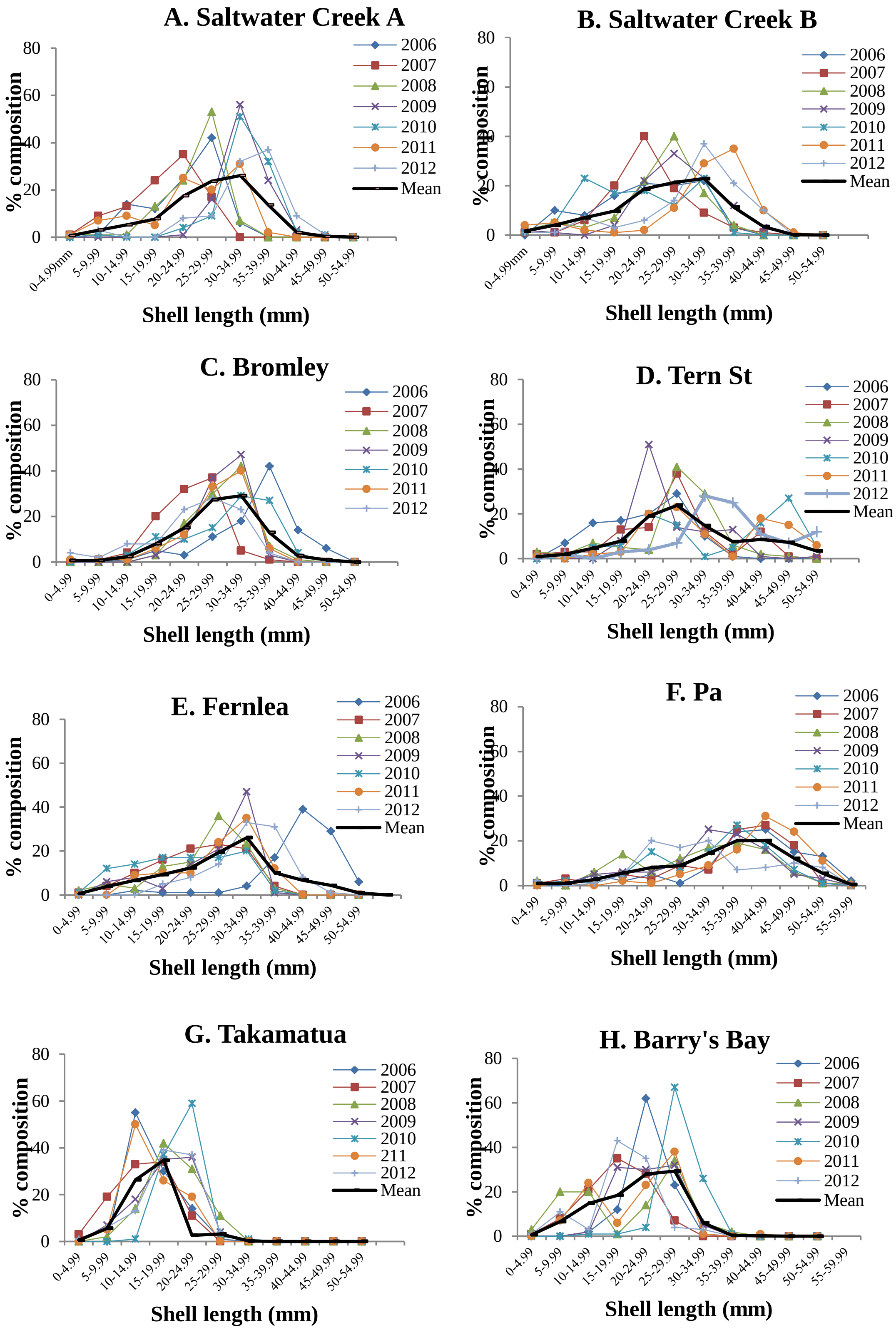


Figure 3.6: Population class division frequencies based on shell length for the 8 Canterbury sites for the 7 summer surveys (2006-2012).

Population density was consistently low at the higher salinity Pa site, Port Levy/Koukourārata (Figures 3.1C & 3.2C) although there was an increase apparent at the 2012 survey (Figure 3.2C). Larger class sizes dominated at this site with few recruits (Figures 3.3F & 3.4F). The site at Takamatua (lower salinity) (Fig 3.3H) with an intertidal length in excess of 300 m had high concentrations of small (<18mm) cockles.

The population structure over the seven summer surveys (Figure 3.5A-H) showed marked differences between the Pa site (Figure 3.5F), with the modal cockle length falling in the range of 30-45mm range and Takamatua (Figure 3.5H), where most cockles were 10-20 mm long. At the other sites the modal cockle length was in the 20-35mm range although there was a shift at Tern Street (Figure 3.5D) in 2012 to 30-40mm. Mean annual shell length (Figure 3.4) showed all the sites to have a uni-modal structure apart from the Pa site which was bi-modal with peaks at 20-15mm and 35-45mm lengths. Similar plots using the summer data only shows all sites to be uni-modal (Figure 3.6). Analysis of the population structure in terms of proportions of recruits (<5mm in length) to mature adults (>20mm) and harvestable sized adults (>30mm) showed that the number of recruits was less than 10% of the population in both the seasonal (Figure 3.7) and annual (Figure 3.8) surveys.

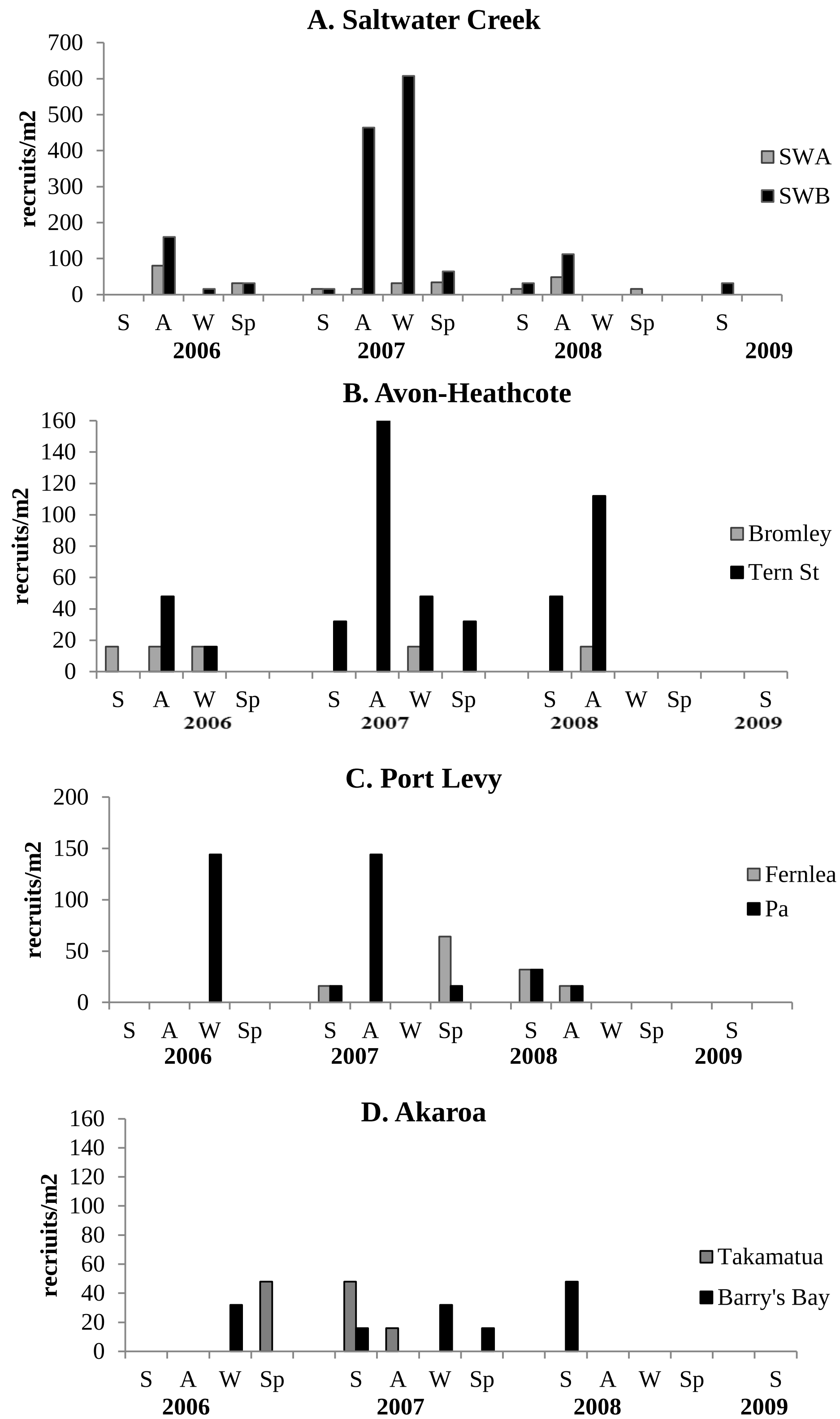


Figure 3.7: Histograms illustrating the number of recruits (<5mm), at all sites for the 13 seasonal surveys. Dark bars identify high salinity sites. NB- y axis values vary.

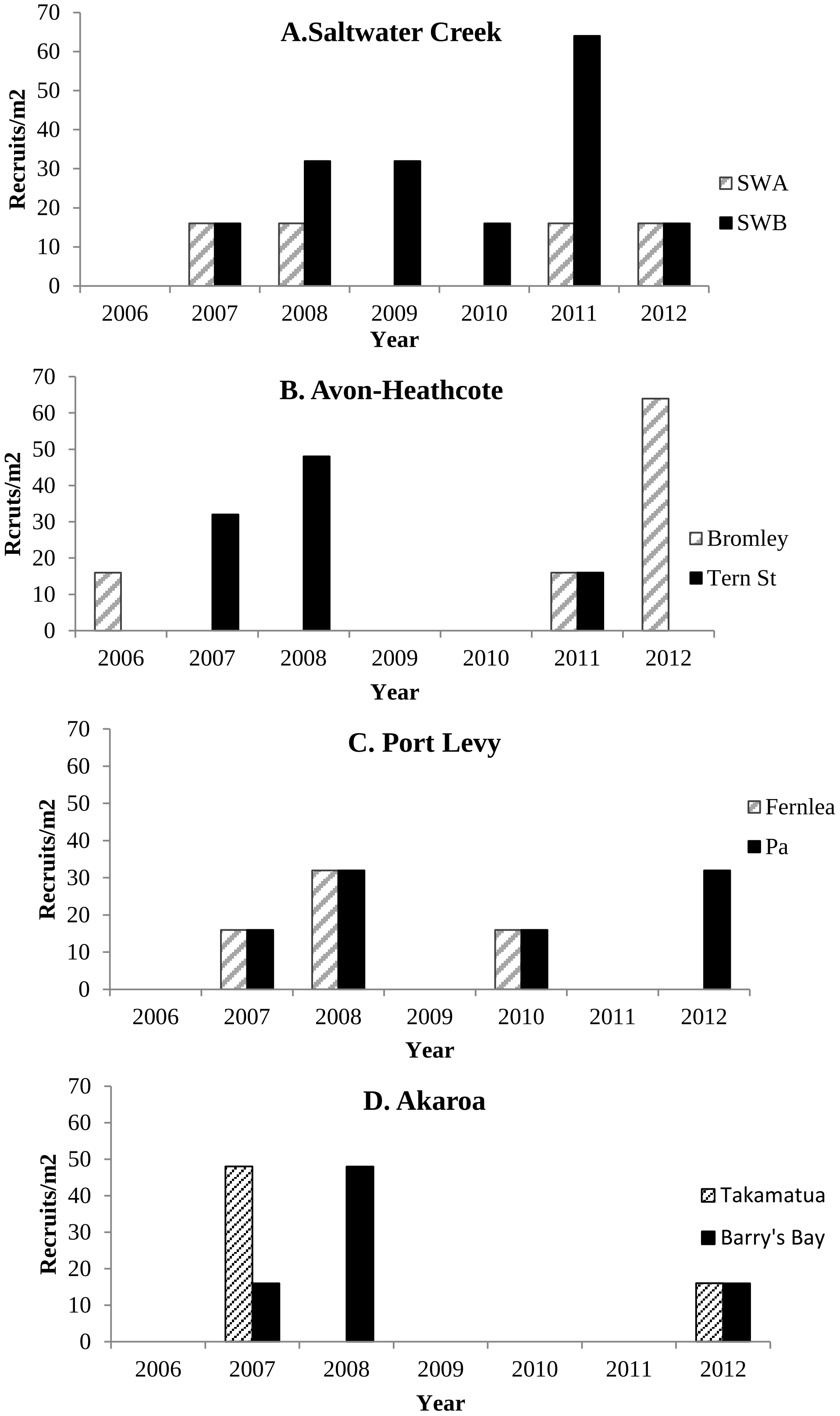


Figure 3.8: Histograms illustrating the proportions of recruits (5mm), at all sites for the 7 summer surveys. Dark bars identify high salinity sites.

Particle size

Sediment structure was predominately a mix of sand and silt (Figure 3.9; Table 3.5), with no significant difference in sediment between the low and high salinity sites.

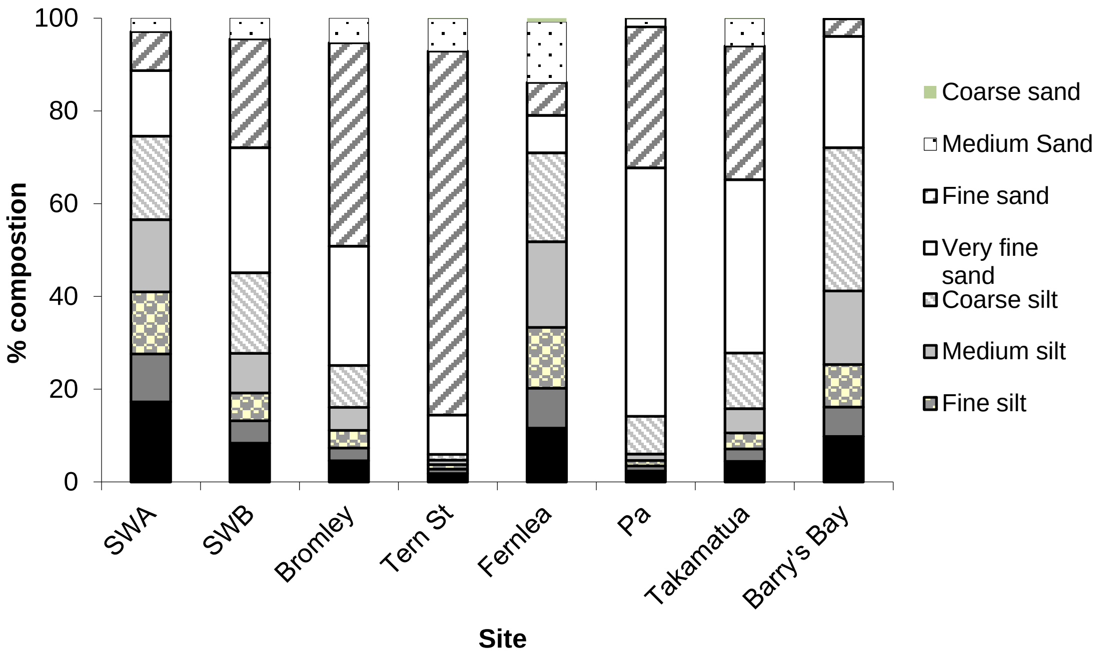


Figure 3.9: Histogram showing sediment % grain composition for the 8 study sites. SWA= Saltwater Creek A; SWB = Saltwater Creek B.

Table 3.5: Mean sediment particle size (microns) and SE (n =3). Shaded cells identify low salinity sites.

	Saltwater Creek A	Saltwater Creek B	Bromley	Tern St	Fernlea	Pa	Takamatua	Barry's Bay
Mean	58.5	56.4	64.7	97.1	88.1	86.2	81.1	45
SE	8.04	7.3	8.3	12.2	10.3	10.3	10.8	5.1
% sand	25.5	64.3	73.9	90.6	39.5	84.9	72.4	29.7
% silt	74.5	35.7	26.1	9.4	60.5	15.1	27.6	70.3

The sites differed in sediment structure (Figure 3.9; Table 3.5) with Barry's Bay having the smallest mean particle size(45 microns) (silt) and Tern Street the coarsest (97.1 microns) (sand).

Sediment trace elements

There were differences between all the sites in the sediment trace element levels although none of the values exceed environmental guidelines (Table 3.6). Mean arsenic concentrations ranged between 2.63 to 15.13ppm, and cadmium from 0.017 to 0.163 ppm. Mean value ranges for chromium were 12 to 28.13 ppm, copper 2.97 to 14.03 ppm and nickel 7.83 to 25.23 ppm, while lead and zinc ranged from 4.94 to 15.57ppm and 28.37 to 69.57 ppm respectively. The metal pollution index (MPI) results (Figure 3.10) identified the Fernlea (Port Levy/Koukourārata) site as the most polluted with the highest values for four (As, Cu, Ni and Zn) of the seven trace elements assessed (Table 3.6). The other Port Levy/Koukourārata site had the highest values for chromium and lead with Tern Street being identified as the least polluted site.

The higher nitrogen levels (Table 3.6) were found at the rural sites (Saltwater Creek, Akaroa Harbour and Fernlea) and at the Avon-Heathcote Estuary Bromley site (site of discharge for waste water treatment). The highest phosphorus level was recorded at Tern Street.

Table 3.6: Mean (±SE) trace element concentrations and nutrients in sediments (ppm) from 8 Canterbury sites. Highest values for each element are in bold. Shaded cells are low salinity sites.

Site	N	P	As	Cd	Cr	Cu	Ni	Pb	Zn
Saltwater A	**1.267 (0.033)**	628 (36.75)	4.8 (0.436)	0.03 (0.003)	13.7 (0.611)	7.77 (0.418)	11.87 (0.537)	11.43 (.0376)	43.53 (1.33)
Saltwater B	0.6 (0.1)	427.33 (23.81)	3.72 (0.267)	0.03 (0.003)	12 (0.436)	6.63 (0.664)	10.53 (0.318)	9.143 (0.806)	37.3 (2.363)
Bromley	0.567 (0.033)	437 (13.65)	2.63 (0.067)	**0.163 (0.025)**	20.5 (0.819)	8.4 (0.306)	9.867 (0.57)	12.27 (0.219)	67.3 (3.378)
Tern St	0.2 (0)	**3311.67 (8.819)**	3.23 (0.24)	0.017 (0.003)	8.8 (0.361)	2.97 (0.186)	7.37 (0.353)	6.397 (0.289)	28.37 (1.132)
Fernlea	0.8 (0.3)	1730.33 (424.68)	**15.13 (4.146)**	0.053 (0.003)	21.63 (2.298)	**14.03 (1.72)**	**25.23 (4.199)**	8.7 (1.809)	**69.57 (4.493)**
Pa	<0.05 (0)	668.33 (17.14	8.8 (0.173)	0.06 (0.006)	**28.13 (4.011)**	8.27 (0.498)	11.4 (0.781)	**15.57 (1.225)**	59.9 (5.129)
Takamatua	0.5667 (0.033)	1333.33 (150.7)	8.77 (0.75)	0.077 (0.003)	15.1 (0.533)	8.77 (0.578)	10.57 (0.664)	4.94 (0.095)	51.93 (3.725)
Barry's Bay	0.9667 (0.067)	832 (145.85)	6.47 (0.612)	0.057 (0.003)	12.7 (0.95)	7.07 (0.578)	7.83 (0.578)	7.993 (0.368)	45.3 (3.467)
ISQG* low-high trigger values (ppm)			20-25	1.5-10	80-370	65-270	21-52	50-220	200-410

*ANZECC Interim sediment quality guidelines (Stewart 2005)

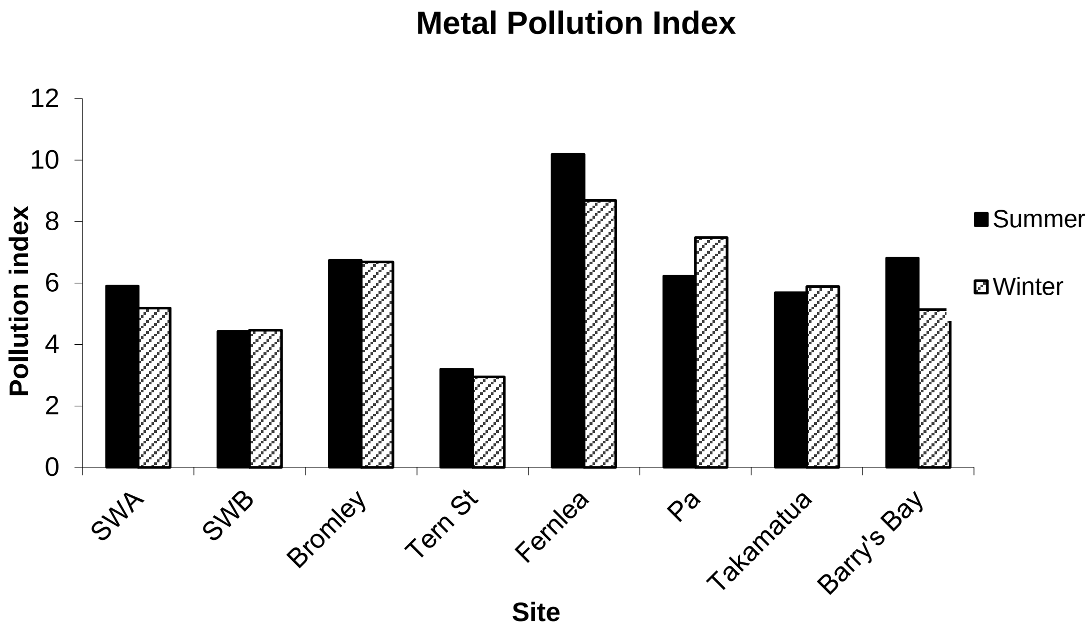


Figure 3.10. Metal pollution indices for surface sediments from the 8 study sites.

Temperature

Monthly mean water and air temperatures showed seasonal variations (Figure 3.11 A-G). Temperatures were uniform across all sites with mean water temperatures ranging from 8°C (winter) at all sites to 20°C (summer) at Fernlea and Barry's Bay. Mean air temperatures were between 6 and 18°C with extremes falling between below zero to in excess of 30°C.

Summer water temperature showed little variation across the sites (Figure 3.12), with mean temperatures falling between 12 and 14°C. Fernlea and Barry's Bay showed the greatest range of summer water temperatures and Tern Street the smallest. Barry's Bay, along with the Pa site, also showed the greatest extremes of air temperature (-2°C to over 32°C). Due to the loss of a data logger at Saltwater Creek, only site B was monitored there.

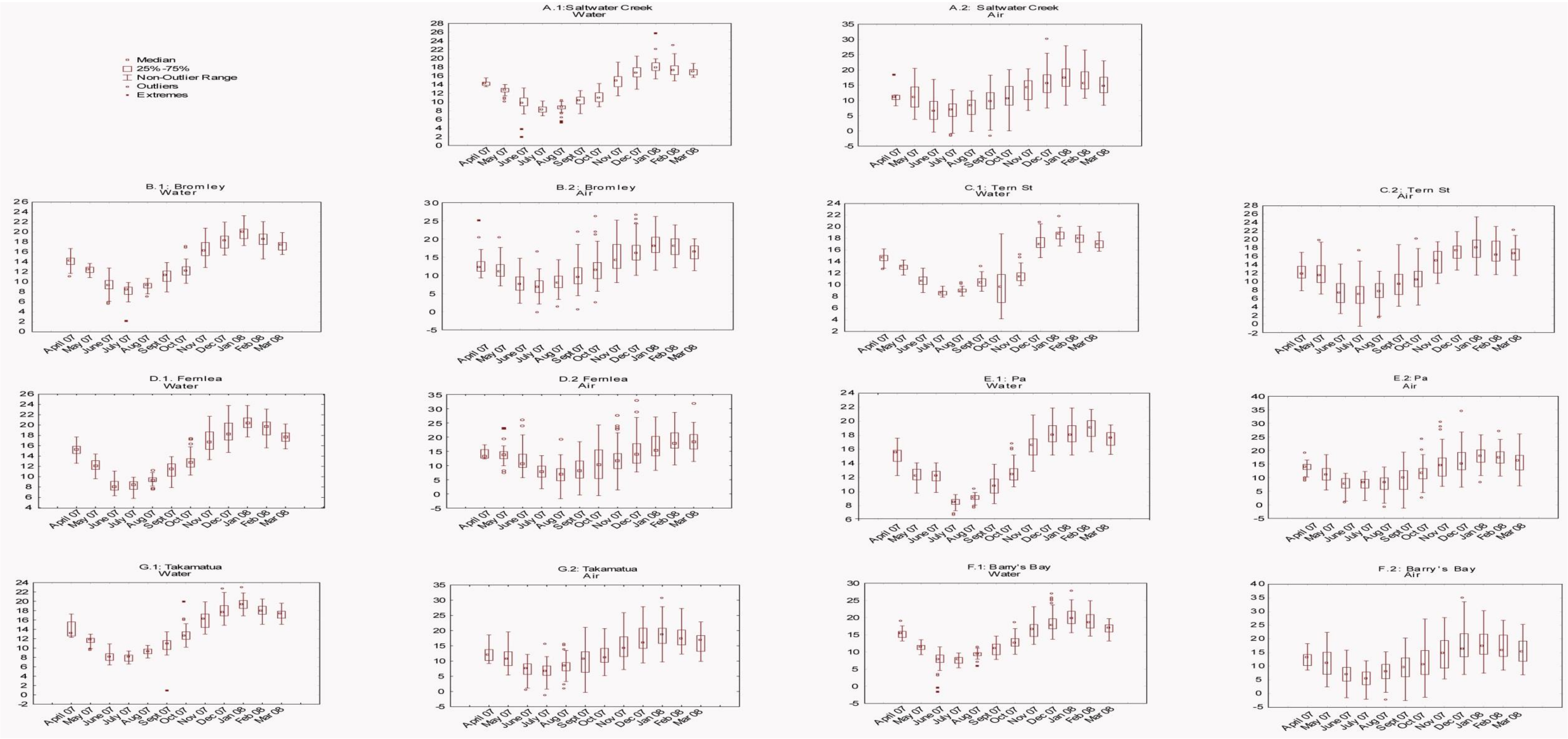


Figure 3.11: Box plots of mean monthly water and air temperatures for 7 study sites. NB. y axis scale is not uniform.

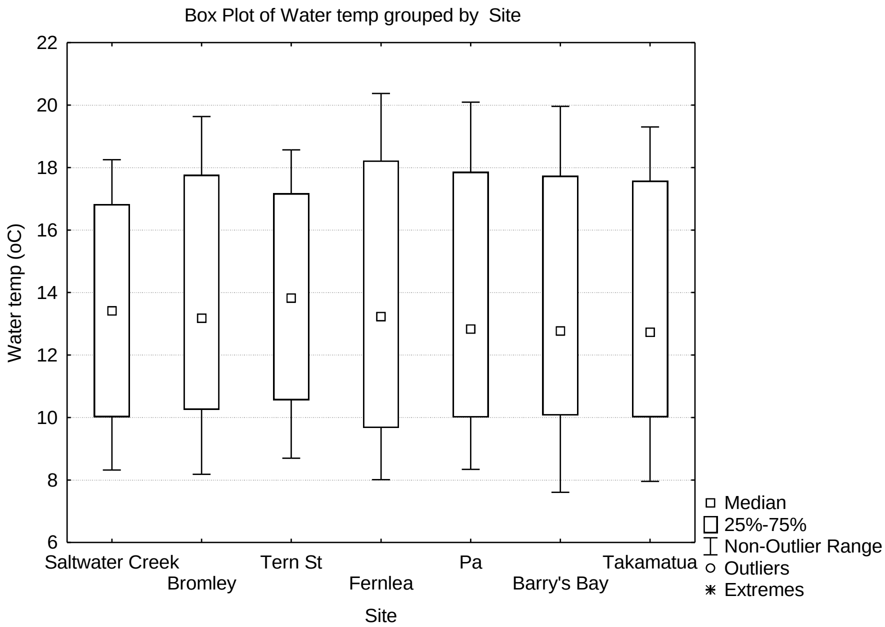


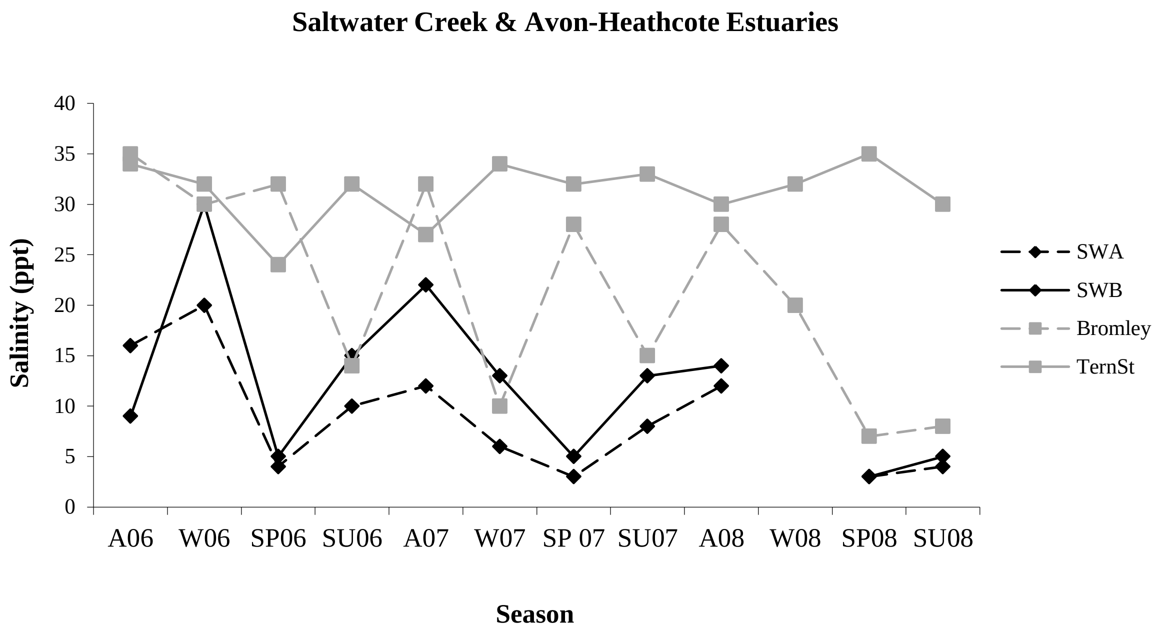
Figure 3.12: Box plot of the mean summer water temperatures for 7 sites.

Salinity

Salinity levels showed differences between the four study areas as well as significant differences between three of the four paired sites (Table 3.7). Tern Street, Pa and Barry's Bay sites had the least variation in salinity with levels in the region of 30-35ppt (Figure 3.13). Low salinity levels (<20 ppt) were found at cockle beds lying adjacent to consistent freshwater inputs. The Bromley site is immediately downstream from the outfall for the waste water treatment plant which discharged low salinity water on each ebb tide. Both Saltwater Creek sites lay alongside the river, and the Fernlea, and Takamatua sites were adjacent to the mouths of streams.

Table 3.7: Single factor ANOVA results describing salinity differences at the paired sites. Significant values are shown in bold.

Area	*df*	*MS*	*F value*	*p-value*
Saltwater Creek	1	58.91	1.18	0.29
Avon-Heathcote	1	560.7	9.54	**0.005**
Port Levy	1	2926	54.8	**0.001**
Akaroa Harbour	1	900.4	8.72	**0.007**



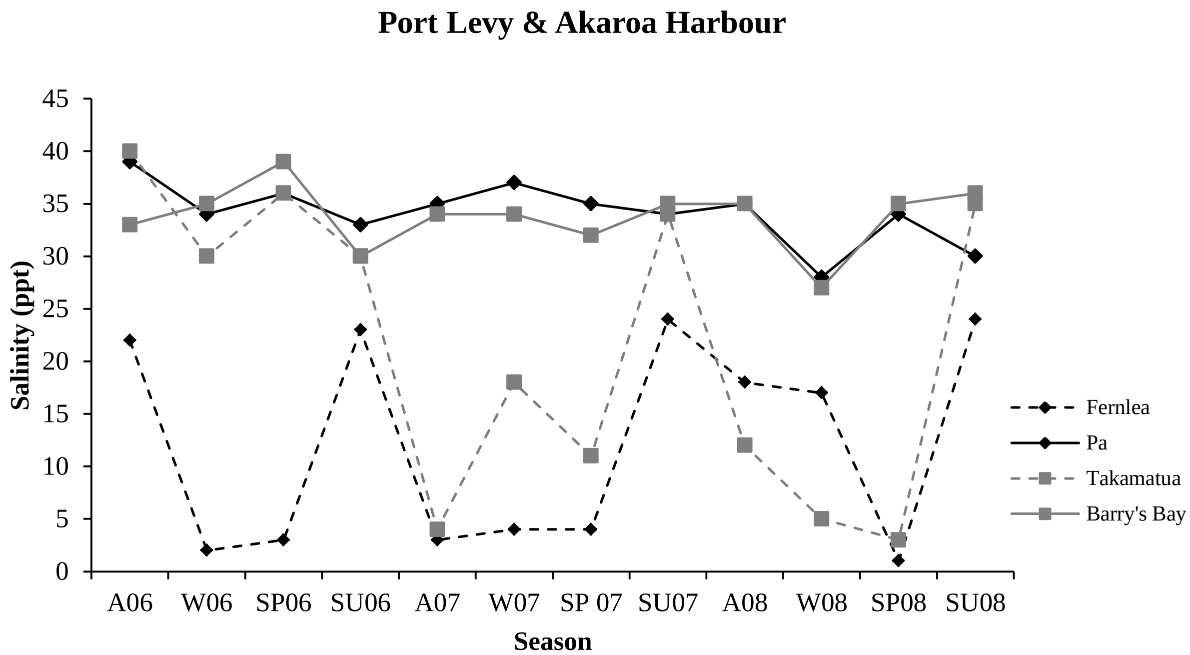


Figure 3.13: Salinity levels (ppt) for (A) the sites within the two estuaries and (B) for sites on Banks Peninsula. (A=autumn, W= winter, SP= spring, SU=summer). Dashed lines identify low salinity sites.

Intertidal length, land use and human impacts

The Avon-Heathcote/Ihutai Estuary had the longest intertidal lengths (600-650 m) and the surrounding land was used for urban development (Table 3.8) and is the site of discharge for the waste water treatment plant for Christchurch city. Fishing and recreational pressure is higher at the more accessible Tern Street site. Barry's Bay and Takamatua had the second longest intertidal lengths (300-350 m) and the surrounding land is used for farming and small settlements. The cheese factory at Barry's Bay discharges water into the bay.

Table 3.8: **Environmental variables for 8 Canterbury sites (ranks 1=lowest impact, 8= highest). Shaded cells indicate low salinity sites.**

Site and human impacts rank	GPS coordinates	Sediment	Salinity Ppt ±1SE	Mean summer water temp°C ±1SE	Intertidal length (m)	Land use and agricultural impacts rank
Saltwater Ck A 2	S43° 16.056' E172° 43.024'	Sand/silt	12 (1.7)	18.43 (0.19)	60-70	Farming 8
Saltwater Ck B 1	S43° 16.135' E172° 43.292'	Sand/silt	15 (2.5)	18.34 (0.19)	50-60	Farming 7
Bromley 6	S43° 32.488' E172° 43.279'	Sand/silt	30 (2.9)	20.13 (1.2)	600-650	City 2
Tern St 8	S43° 33.229' E172° 44.537'	Sand	32 (0.9)	18.83 (0.11)	600-650	City 1
Fernlea 4	S43° 39.637' E172° 49.093'	Sand/silt, on larva base	2 (2.9)	20.4 (0.17)	40-50	Farming 5
Pa 7	S43° 39.171' E172° 49.868'	Sand	34 (0.84)	20.4 (0.13)	30	Farming 6
Takamatau 5	S43° 46.893' E172° 57.805'	25% silt	3 (4.1)	19.29 (0.18)	300-350	Farming 3
Barry's Bay 3	S43° 45.752' E172° 54.875'	95% silt	35 (0.9)	19.6 (0.33)	300	Cheese factory, Farming 4

The intertidal lengths at Saltwater Creek fall between 40-50 m with the surrounding land being used for mixed farming. The shortest intertidal lengths were at Port Levy/Koukourārata; 40-50 m at Fernlea and 30 m at the Pa site. This last site has historically been subject to high human impacts as it lies alongside the main roadway and across from the settlement. Both areas are surrounded by farmland.

Microorganics

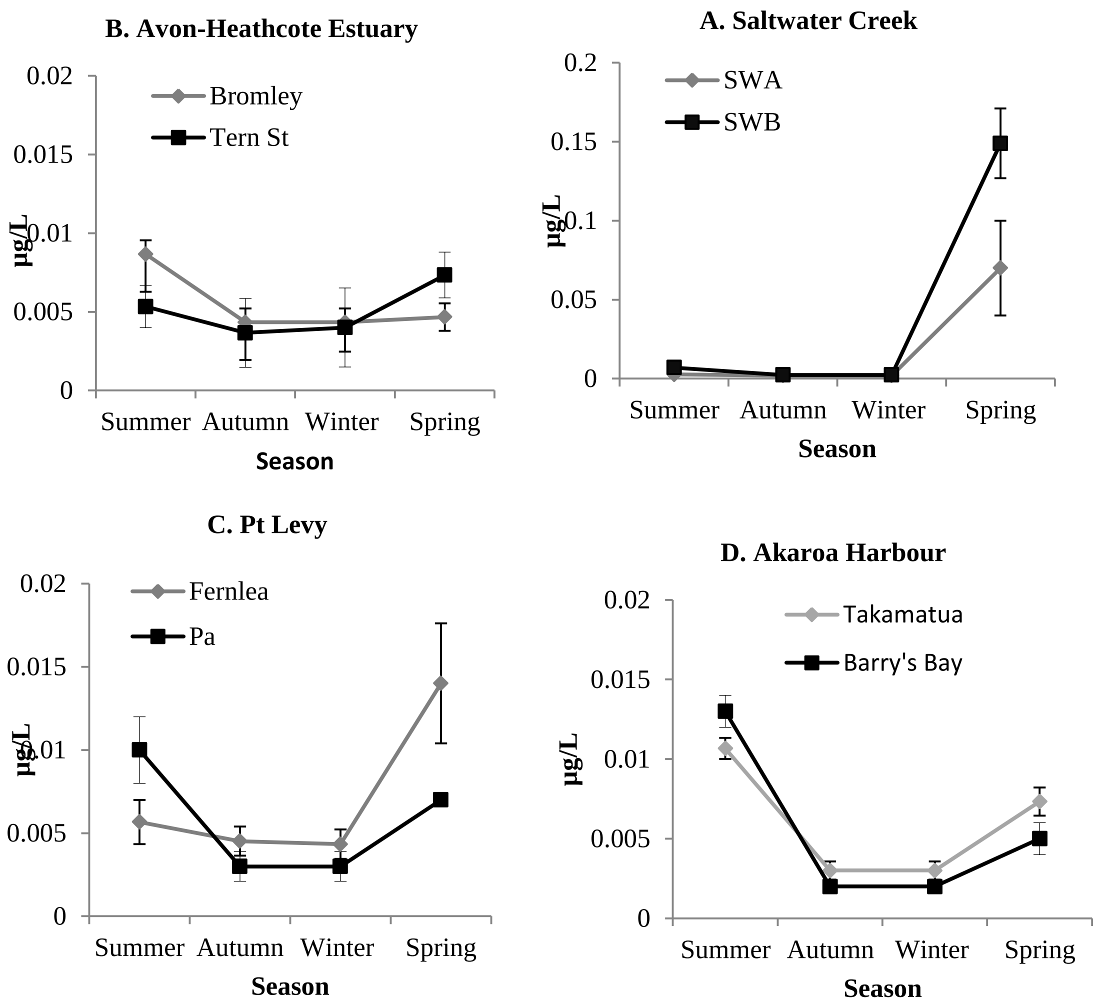


Figure 3.14 A-D: Mean seasonal total volatile solids (mg/L) ±1SE for the 8 study sites. Dark lines identify higher salinity sites.

Total volatile solid (TVS) levels (Figure 3.14A-D) had similar low levels (Table 3.7) across all sites (generally 0.01mg/L or less) except for Saltwater Creek (Figure 3.14A) which showed higher values at the spring sampling time. All site sediments had lower values over the autumn/winter months.

Table 3.9: ANOVA results comparing mean seasonal TVS between high and low salinity sites.

Source	*df*	*MS*	*F*	*P-value*
Season	3	0.001647	2.375337	0.098957
Sites	7	0.000615	0.886591	0.53393
Error	21	0.000693		
Total	31			

Correlations

Correlation analysis (Table 3.10) showed water temperature was positively correlated with mean cockle length (0.896) (p= <0.5) and largest size (0.884). Shell length being positively correlated with increased water temperature can be explained by warmer conditions enhancing filtration rates, optimising nutrient intake. There were no significant correlations of population characteristics with salinity with only a significant negative one with nickel. No significant correlation between size and density supports the lack of evidence of density limiting mean size. Some trace metals and nutrient levels were positively correlated (As 0.953; Cu 0.839: Ni 0.781) with a negative correlation between density and lead (-0.952). MPI showed a significant correlation with air temperature (0.929), an unexpected outcome, and a negative correlation with TVS (-0.78) which may be explained by the presence of metal pollutants impacting on the availability (i.e. growth) of nutrients. TVS was negatively correlated with air temperature (-0.841) but not to water temperature.

Table 3.10: Results of Spearman Rank Correlation analysis investigating the relationships between cockle size, density, trace metals and environmental variables.
Correlations in bold are significant (*p*=<0.5)

	Site	*Mean size*	*Largest size*	*Density*	*Total N*	*P*	*As*	*Cd*	*Cr*	*Cu*	*Ni*	*Pb*	*Zn*	*salinity*	*%silt*	*%sand*	*Temp H2O*	*Temp air*	*Mean MPI*	*TVS*
Site	1																			
Mean size	-0.275	1																		
Largest size	-0.167	**0.923**	1																	
Density	0.239	-0.591	-0.429	1																
Total N	-0.181	-0.466	-0.676	-0.088	1															
P	0.534	-0.165	-0.271	0.111	0.372	1														
As	0.505	0.109	0.05	-0.089	0.204	**0.935**	1													
Cd	0.122	0.19	-0.066	-0.291	-0.097	0.006	-0.11	1												
Cr	0.228	0.685	0.545	-0.575	-0.309	0.347	0.515	0.452	1											
Cu	0.189	0.169	-0.076	-0.349	0.399	**0.839**	**0.828**	0.286	0.618	1										
Ni	-0.001	0.289	0.119	-0.235	0.334	**0.781**	**0.839**	-0.042	0.451	0.886	1									
Pb	-0.333	0.777	0.657	**-0.783**	-0.258	-0.284	-0.06	0.278	**0.722**	0.157	0.055	1								
Zn	0.237	0.434	0.156	-0.494	0.063	0.565	0.581	0.688	**0.841**	**0.842**	0.616	0.431	1							
salinity	0.059	0.075	0.342	0.024	-0.612	-0.289	-0.157	-0.011	0.068	-0.500	**-0.721**	-0.645	-0.197	1						
%silt	-0.159	-0.309	-0.494	-0.302	**0.952**	0.344	0.269	-0.182	-0.16	0.418	0.352	-0.053	0.094	-0.465	1					
%sand	0.159	0.309	0.494	0.302	-0.952	-0.344	-0.27	0.182	0.161	-0.418	-0.35	0.053	-0.094	0.465	**-1**	1				
Temp H2O	0.007	**0.896**	**0.884**	-0.332	-0.607	0.086	0.336	0.161	0.68	0.259	0.423	0.489	0.472	0.188	-0.506	0.506	1			
Temp air	0.585	0.233	0.114	-0.088	-0.115	0.4	0.392	0.562	0.365	0.354	0.252	-0.109	0.587	0.387	-0.143	0.143	0.428	1		
Mean MPI	0.444	0.542	0.448	-0.203	-0.335	0.337	0.433	0.489	0.554	0.373	0.363	0.118	0.636	-0.291	-0.315	0.315	0.7325	**0.929**	1	
TVS	-0.621	-0.225	-0.159	-0.116	0.174	-0.313	-0.31	-0.376	-0.34	-0.146	-0.05	0.04	-0.403	-0.546	0.175	-0.175	-0.352	**-0.841**	**-0.78**	1

Discussion

Population density of *Austrovenus stutchburyi* was variable both between the survey areas and within the paired sites, as has been found in cockle populations from other parts of the world. These global variations in population abundance and structure have been linked to human impacts (Carmichael et al 2004; Harding 2007; Johnson & Heck 2007; Atkinson et al. 2010), environmental factors (Bouma et al. 2001; Huxham & Richards 2003; Hofman et al. 2006; Hermann et al. 2009; Lundquist et al. 2009; Powers 2009; Perrigault et al 2010), nutrient availability and recruitment success (Flach 1996; Richards et al. 2002; Huxham & Richards 2003; Strasser et al. 2003; Bos 2006; Dethier 2010; Genelt-Yanovskiy et al. 2010). In the present study no single environmental factor explained variation in population density and structure suggesting that multiple stressors including nutrient levels are responsible for the major differences.

Intertidal communities are typically patchy and expected to vary on different scales (Thrush et al. 2003) both within and between estuarine systems. The present study found more population variation between estuaries than seasonally within estuaries. Similar findings have been found for benthic assemblages in Tasmanian estuaries (Edgar et al. 2000; Edgar & Barrett 2002; Hirst & Kilpatrick 2007).

Population density of *Austrovenus stutchburyi* varied between the estuarine systems (Figures 3.1 A-D and 3.2 A-D; Tables 3.1 and 3.2) with densities varying between the paired high and low salinity sites (Tables 3.1 and 3.2) except for the sites at Saltwater Creek, where the salinity levels were more similar than for the paired sites at the other areas.

In the Saltwater Creek Estuary, density at both sites was consistently less than 800 cockles /m^2, although there was a definite increase at Site A following a major flooding incident in the winter of 2008 (Fig.3.1A).

The two sites at the Avon-Heathcote/Ihutai Estuary showed considerable differences in density (Fig 3.1B). Bromley was consistently low, less than 400 cockles /m^2 whereas Tern Street frequently had densities greater than 1200 cockles /m^2. In this estuary both populations appear to be stable with juvenile and large adult cockles present. The mean densities of *A.stutchburyi* occurring in the Saltwater Creek and Avon-Heathcote/Ihutai estuaries were on a par with those occurring at Tamaki estuary, an impacted estuary in the North Island of New Zealand (Stewart 2005). Densities there were 997/m^2 in 1990 (Bioresearches 1990) and 708/m^2 in 2005 (Stewart 2005). These results all demonstrate that populations of the New Zealand cockle can become established and survive in contaminated sediments.

Cockle densities at the Pa site (Port Levy/Ihutai) were consistently low (<200 cockles/m^2) a reflection of the status of this area which is under the protection of a rahui controlling the harvesting of these bivalves. This bed had a harvesting ban for over seven years prior to the beginning of this study and in recent years limited gathering has been allowed under permit for two weekends in September in an endeavour to restore the beds to their once bountiful level. Unfortunately, in modern times the traditional harvesting practises have not been adhered to, and as this bed is easily accessible it has been exploited. At Fernlea the densities varied between 200- 500 cockles/m^2. This area also comes under the rāhui, but the lower cockle densities are not due to overharvesting, but from environmental factors. Episodes of heavy rain cause flooding of the adjacent waterway, with the increased flow scouring out the cockle bed.

With water runoff comes sediment deposition from the surrounding land, and this silt accumulates at the top of the inlet, impacting on the filtering efficiency of the cockles.

In Akaroa Harbour there was a difference in cockle abundance between the high-salinity Barry's Bay site (<500 cockles/m^2) and the low-salinity Takamatua site (1500-2500/m^2). This is at odds with the concept that salinity is a controlling factor in population structure but suggests that multiple factors play a part. At the top of the harbour, Barry's Bay densities are influenced by silty sediment depositions while Takamatua is probably more controlled by salinity levels. At this site, it is not low numbers that are an issue, but lack of growth in the cockles.

Cockle densities in the present study were highest (>1200 cockles /m^2) at Takamatua (Akaroa Harbour) and Tern Street (Avon- Heathcote/Ihutai Estuary), one relatively uncontaminated site and one degraded site. In the present study, Fernlea in Port Levy was identified as the most contaminated site but maintained higher cockle densities. The source of the higher levels of trace elements may be the weathering of the volcanic rock that makes up Banks Peninsula, and the size and density of the cockles may depend on the nutrient inputs from the adjacent stream.

Within the Avon-Heathcote, lower densities (< 400/m^2) were maintained at a site close to a waste water treatment plant. While this site has high salinity levels at high tide (30g/100g) on the ebb tide these would drop due to large inputs of fresh water discharged from the oxidation ponds. The siltier substrate of this site could also impact on the feeding efficiency of the cockles, with finer particles more readily suspended in the water column. Lowest cockle densities in the present study were found in Port Levy at the Pa site (<100/m^2) and were consistent with results from annual surveys conducted since the imposition of a rāhui (a restriction on the access to, or use of, an area or resource by unauthorised persons) in 1996 (Voller 2003 ; The Press, 1996; Marsden and Adkins 2010).

As in many other infaunal bivalves (Flach 1996; Jayawickrema & Wijeyaratne 2009; Genelt-Yanovskiy 2010), size class distributions of the New Zealand cockle were variable both amongst and within sites representing the stability of a population as well recruitment episodes. Cockle population structures in the Canterbury area were site specific (Figures 3.3, 3.4, 3.5.and 3.6; Table 3.3), with irregular recruitment patterns and some sites dominated by small animals and others consisting mostly of very large breeding sized individuals (Figures 3.7 and 3.8) (Larcombe 1971; Stewart 2005). The low numbers of small (juvenile) individuals was a common factor supporting the picture of irregular recruitment and populations in decline (Stewart 2005). At the sites where larger cockles were present, for example Pa site, Port Levy/Koukourārata, the overall density and recruitment of these bivalves was low suggesting that bivalve density is important in providing physical stability for beds, thus providing secure settling sites for spat.

Within the Saltwater Creek Estuary, the lower salinity site was dominated by adult cockles. The flood which closed the adjacent main highway caused some scouring in this cockle bed, and small cockles may have been swept away. In terms of class size proportions, the flooding event during the winter of 2008 was followed by a change in population structure with a greater percentage of large cockles present. The more marine site, closer to estuary mouth, also had a definite adult population but large peaks of small individuals were found at regular intervals suggesting that recruitment was occurring.

In the Avon-Heathcote/Ihutai Estuary, the low-salinity Bromley site had lower cockle recruitment success than the high-salinity Tern Street site. The timing of recruitment differed between the two sites, with the Bromley site showing a small influx of juveniles over autumn/winter of 2006. Two peaks showed at Tern Street; one between autumn and spring of 2007 and the second during the autumn of 2008. Both populations appear to be stable with both juveniles and large adults present. The high density of larger cockles at Tern Street which had the longest intertidal length is probably related to the nutrient quality as this estuary is the discharge site of the water treatment plant for the adjacent city. The increase in the number of cockles greater than 50mm in length at the 2012 survey could possibly be attributed to effects of the earthquakes which commenced in September 2010. Due to the resulting damage to waste water infrastructures, it was necessary to discharge raw sewage directly into either the estuary or into the rivers feeding into it, increasing the amount of nutrients available.

Within Port Levy/Koukourārata, on Banks Peninsula, at the Pā site, where population density was consistently low (48 per m^2), larger class sizes dominated with few recruits. Stewart (2005) describes a population with similar characteristics as a remnant population. The Pā population occurs within a Maori reserve, where a rahui (harvesting ban) was established to restore customary mahinga kai (customary and recreational food gathering). Although collecting has been prohibited since 1997, recruitment has continued to be irregular. The reasons for this are not obvious; perhaps there is a lack of spat or the low density of large adults does not aid the successful settlement as in some other bivalves (Defeo et al 1992; Marsden 2002). This estuary has a long narrow inlet and therefore lacks good connectivity with adjacent oceanic currents. The inlet also includes a mussel farm with lines which may have unknown effects on infaunal bivalves and their recruitment.

Barry's Bay, the higher salinity site in Akaroa Harbour presented as a stable population with a range of length sizes, supporting the occurrence of recruitment. The low salinity site at Takamatua had very high densities of moderately small cockles. The lack of large (breeding–

sized) cockles does not appear to have an impact on the continuity of this population, a fact supported by histological analysis of the gonads (Growth and reproduction, Chapter 4) from cockles from this site.

Total nitrogen and phosphorous levels were also variable with the highest N levels occurring at both Saltwater Creek sites (rural with run off of farm fertilisers) and at Bromley, the site of discharge for the waste water treatment plant. Tern Street (higher salinity site close to the mouth of the Avon-Heathcote/Ihutai Estuary) shows the lowest phosphorus level, suggesting that this area is well flushed and that phosphorus entering through the waste water system is not accumulating. Sediment element levels (As, Cd, Cr, Cu, Ni, Pb and Zn) for the 8 sites were variable (Table 3.6), with the most contaminated site being Fernlea with the highest concentrations of arsenic, nickel, copper and zinc. High concentrations of zinc and chromium were also found at the Bromley and Pa sites. However, none of the element concentrations exceeded the ANZECC ISQG high trigger values, and only one (nickel at the Fernlea site) exceeded its low trigger value. Based on these findings, the Canterbury cockle beds surveyed for this study cannot be described as showing significant trace metal contamination. The trace metals present can be linked to the weathering of volcanic rocks or to industrial processes which are no longer permitted to discharge their wastes into the waterways.

Salinity variations can be related to the proximity of the beds to freshwater inputs. The sites at Saltwater Creek, Fernlea, and Takamatua are adjacent to quite large freshwater water courses, with the Bromley site lying alongside the outflow of the waste water treatment ponds. As a result of seismic activity beginning in September 2010 in the Canterbury region, there has been variable discharges of (untreated) waste water directly into the Avon-Heathcote Estuary which will have had an effect on the available nutrients and this may go some way to explaining the increased size of cockles at Tern St in the summer of 2012.

Cockle population structures in the Canterbury area were site specific, with populations being highly variable in both class size and density. These variations cannot be explained directly by the measured trace elements (As, Cd, Cr, Cu, Ni, Pb and Zn) and nutrient levels (N and P) or sites ranked according to anthropogenic inputs. Sediment element levels (As, Cd, Cr, Cu, Ni, Pb and Zn) for the 8 sites were variable but did not exceed the NZ and Australian levels for minimal environmental effects.

Some populations continued to thrive in areas that receive contaminants from waste water, with the high density of larger cockles at Tern Street, probably related to the nutrient quality as this estuary has been until recently the discharge site of the water treatment plant for the adjacent city. The low population densities recorded in this present study would not support commercial harvesting as occurs at Whangarei Harbour, Nelson/Marlborough and Otago Harbour with cockles greater than 30mm length being targeted (Morrison & Brown 1999, Cryer et al. 2004, Marsden & Adkins 2009). Cockle growth rates have been related to a number of factors including temperature, salinity, and nutrient resources (Stewart & Creese 2002; Marsden 2004), with faster growth rates in the North Island, and cockles reaching a harvestable size at 2 years of age (Marsden & Adkins 2009). Similar patterns of variability in class size and density between sites and years has been recorded for both cockles and mussels in England and Wales (Dare et al 2004). These variations were linked to the amount of feeding time available as well as to population density. However, in this study, no one environmental factor was identified as having a controlling effect on the populations of cockles monitored in the Canterbury region of

New Zealand. It may well be that there is a cumulative effect of a number of factors which is responsible for the variation between these populations.

Chapter 4

Reproduction and growth

Introduction

All organisms, including bivalves undergo cycles of growth and maintenance, gamete production and other reproduction processes, as well as using energy for metabolic activities. However, the latter may proceed at the expense of the former, and may be attributed to environmental stresses (Guerra et al. 2012). When energy is channelled into the functioning and maintenance of an animal, there may be no surplus for growth and reproduction. Measurement of the accumulation of energy reserves in individual animals can be used to assess the health of a population in terms of its long-term survival (Stevenson & Woods 2006). Low energy reserves may result in reduced gametogenesis and even the demise of individuals.

Glycogen, composed of glucose units, is the carbohydrate storage material found in animals, and measurement of these levels can indicate the amount of stored energy available for activities such as reproduction. In bivalves, seasonal changes in glycogen levels which can be measured from soft tissues including foot muscle and gills have been related to gonad development and subsequent spawning events (Navarro et al. 1989; De-Luca Abbot 2000; Stewart 2005; Liu et al. 2008).

Reproductive cycles in bivalves follow the sequence of growth and maturing of gametes, spawning, and then gonad recovery (Gosling 2003), with variation in timing and length of each stage being species specific and also site specific probably linked to temperature, salinity, light and nutrient availability. It is generally accepted that *Austrovenus stutchburyi* reaches reproductive maturity at 18mm in length (Stewart 2005), but it is also recognised that under stressful conditions or in less than ideal habitats, invertebrates may reach reproductive maturity at a smaller size (Stewart 2005). Reproductive cycles can be correlated with condition index to identify when spawning events may occur.

Condition indices can be used to track seasonal and site differences in nutrient reserves as well as being stress indicators. Crosby & Gale (1990) reviewed condition indices methods and based on statistical comparisons of the three primary formulae recommend the use of the CI gravimetric formula as an index of bivalve nutritive status and recent stress. This formula,

(CI_{grav} = (Dry tissue weight (g) x 1000)/ internal shell cavity capacity (g)

where the internal shell cavity capacity = total whole live weight (g)– dry shell weight (g)) was selected for the study in preference to the CI shell or dry condition ((dry tissue weight/dry shell weight)*100) index used by Roper et al. (1990), Gardner (1993) and Marsden & Pilkington (1995), which while easy to measure, does not make allowance for variations in shell shape or thickness (Norkko 2005). Comparison of condition index using both methods on a representative sample of *Austrovenus stutchburyi* showed less variation in the index values with the CI dry weight formula than with the gravimetric one. This decision was also supported by the findings of Stewart (2005) who found that the dry weight condition index failed to separate cockles from clean and polluted sites i.e. it is not a reliable indicator of environmental stress. Norkko (2005) also elected to use the CI gravimetric index because it compensates for any shell damage caused through predation.

Energy is also used for shell growth, both length and thickness, with faster growing individuals having thinner shells (Watanabe & Katayama 2010). The growth rate of bivalves including various species of venus clams can be variable due to a number of factors such as salinity and nutrient availability (Shriver et al. 2002; Carmichael et al. 2004) as well the presence of endoparasites and attached epibionts (Trigui El-Menif et al. 2008). Age can be assessed by counting external shell growth rings which are laid down annually or by counting growth rings on sectioned shells (Richardson 1987; Kilada et al.2007).

Compromised shellfish health with associated reductions in condition and growth has been related to human induced activities including habitat modification (Johnson & Heck, 2007), industrial contaminants, urban development, land run off and increased sedimentation (De Luca-Abbott et al. 2000, Stewart 2005, Norkko et al. 2006; Hewitt & Norkko 2007; Heggie & Savage 2009; Thelen & Thiet 2009; Jayawickrema & Wijeyaratne 2009) as well as to environmental factors such as temperature (Guerra et al. 2012) and salinity (Marsden & Pilkington 1995; Marsden 2004) .The aim of this facet of the research was to investigate the variability in the growth, and condition of *Austrovenus stutchburyi* at the four estuarine areas studied for population dynamics in the previous chapter. Again, the effects of salinity and sediment, both contaminants and structure, are important considerations, with it being hypothesised that sites with low salinity levels or with contaminated or very fine sediments would have slower growth rates, lower glycogen levels and condition indices and reduced reproductive output. Histological sectioning of gonad tissues allowing the assessment of reproductive condition or state was used to assess the potential for cockle recruitment. As with the population structure investigation, it was predicted that the populations would be variable in reproductive status, glycogen levels, condition and growth due to differences in environmental parameters, and human impacts on the areas. Being a long term study covering pre and post-earthquake events, the results of this research provide a two-pronged approach. Firstly, they are able to be used in evaluating cockle beds in other areas, both in New Zealand and overseas, as well as allowing comparisons with other bivalve populations in the Canterbury area. Secondly, they give a before and after response of natural systems to environmental disruption.

Methods

Reproduction, age and growth

Reproduction

For histological examination, sub-samples of cockles (n=10) were collected at each site during population surveys in April, July and October 2006 and January 2007, and transported to the laboratory chilled, where they were measured and opened and the flesh preserved in Davison's fluid until processing. Samples were processed in the School of Biological Sciences, University of Canterbury. Samples were dehydrated using an ethanol series, blocked in paraffin wax and sectioned to 6-7µm thickness. Once mounted on slides, sections were stained with Haematoxylin and counterstained with Eosin.

Development stage assessment was carried out by random examination of one field of view and assigning the specimen to a development stage as described in Table 4.1. Examples of various development stages (Figure 4.1 A & B) illustrate the classification criteria as set out in Table 4.1. All slides were examined without knowledge of site of origin or time of collection of the cockle.

Microscopic examination was used to determine the number of follicles per view for both male and female specimens. For each female specimen, 10 randomly selected follicles were measured for length using an eyepiece graticule. Both nucleated and non-nucleated ova were counted in two of these follicles, and the diameter of five ova (nucleated where possible) were recorded for each. For each male specimen the length of 10 randomly selected follicles was measured.

Due to differences in cockle size between sites, it was not possible to use cockles of a uniform size for histological processing. Individuals from the Takamatua site were consistently considerably smaller than those from all the other sites. The mean shell length collected for histology across all sites and over all seasons was 27.5mm with a range of 15.5mm (Takamatua) to 51.7mm (Pa) (Table 4.2). The histological sections were also inspected for parasitic infestation of the foot muscle.

Table 4.1: Staging criteria and gonad index values used to determine the reproductive status of *Austrovenus stutchburyi* (Stewart 2005).

Stage	Gonad index	Males	Females
Indeterminate or empty	0	No gametes present to determine sex. Follicles empty or absent. Connective tissue present.	No gametes present to determine sex. Follicles empty or absent. Connective tissue present.
Early active	3	Thin follicle walls. Spermatocytes and spermatids present in roughly equal amounts. A few free sperm in lumina.	Small gonad area. Thick follicle walls with oocytes attached by stalk or broad base. Nucleus fills most of cell
Late active	4	Larger follicles. Fewer spermatocytes and spermatids. Many mature sperm	Thinner follicle walls. Many free oocytes and ova. Attached oocytes have thin stalk. Some spaces in lumen
Ripe	5	Profuse sperm. Follicle walls thin, spermatogonia region not clearly distinguishable.	Thin follicle walls. Many ova in lumina. Little ova generation apparent.
Spawning	2	Density of sperm in lumina variable with many gaps.	Many lumina have spaces and thin walls. Reduced numbers of ova. Some follicles ruptured.
Spent	1	Small gonad volume, thick follicle walls and connective tissue present. Very few sperm.	Reduced follicle size. Connective tissue present. Very few ova or mature oocytes present.

Table 4.2: Mean shell length (mm) and length range of cockles selected for histological examination of reproductive stages from the 8 Canterbury sites. Shaded cells identify high salinity sites

Site	Mean (mm)	Range (mm)
Saltwater Creek A	24.7	18.0-32.4
Saltwater Creek B	27.3	20.4-38.8
Bromley	29.0	21.8-40.5
Tern Street	30.2	21.4-39.7
Fernlea	30.9	19.9-38.8
Pa	35.6	18.8-51.7
Takamatua	20.3	15.5-27.4
Barry’s Bay	21.4	17.5-39.1

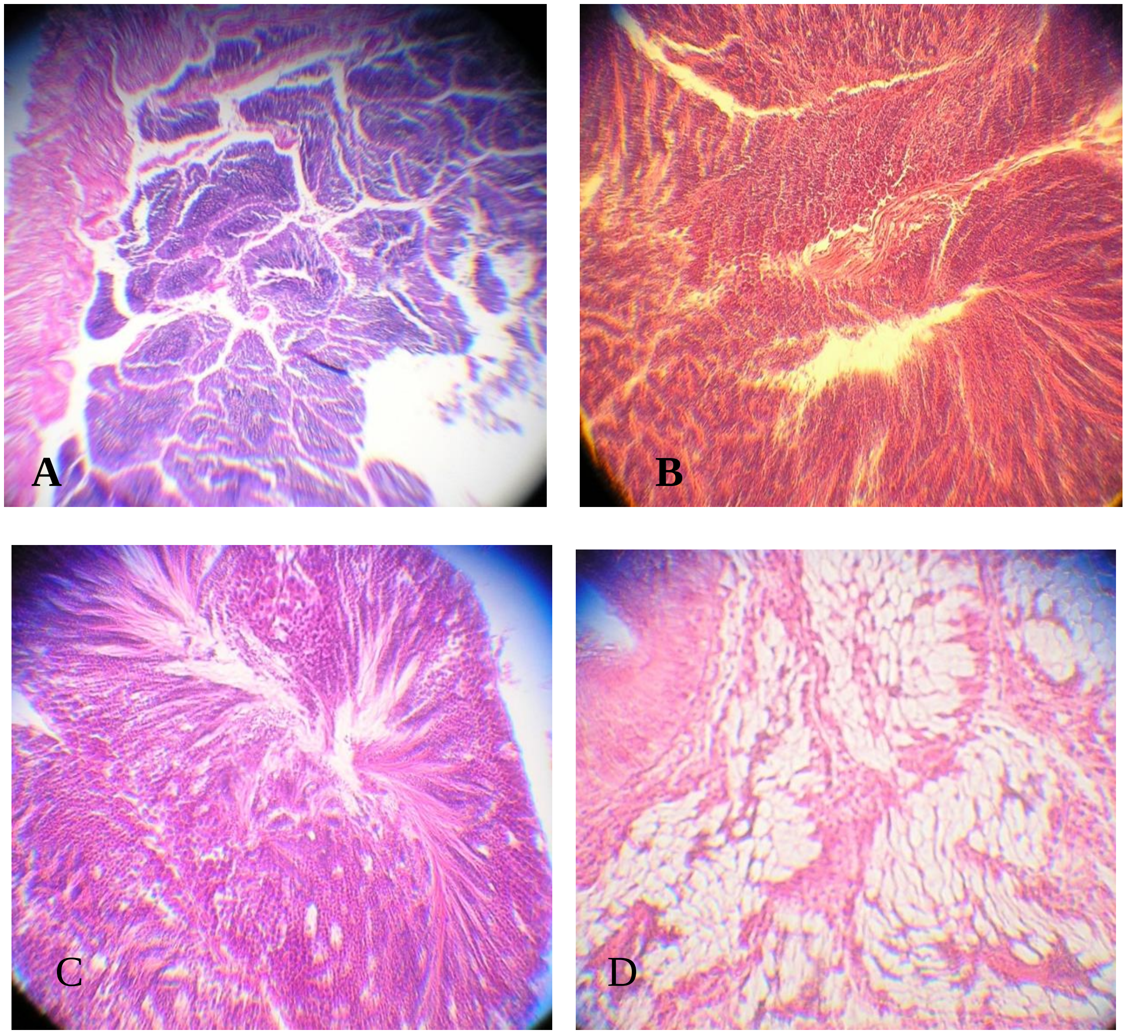


Figure 4.1A: Photographs of reproductive stages of male *Austrovenus stutchburyi.* Males (A) early active, (B) ripe, (C) spawning, (D) spent. All photographs are viewed at X 40 magnification.

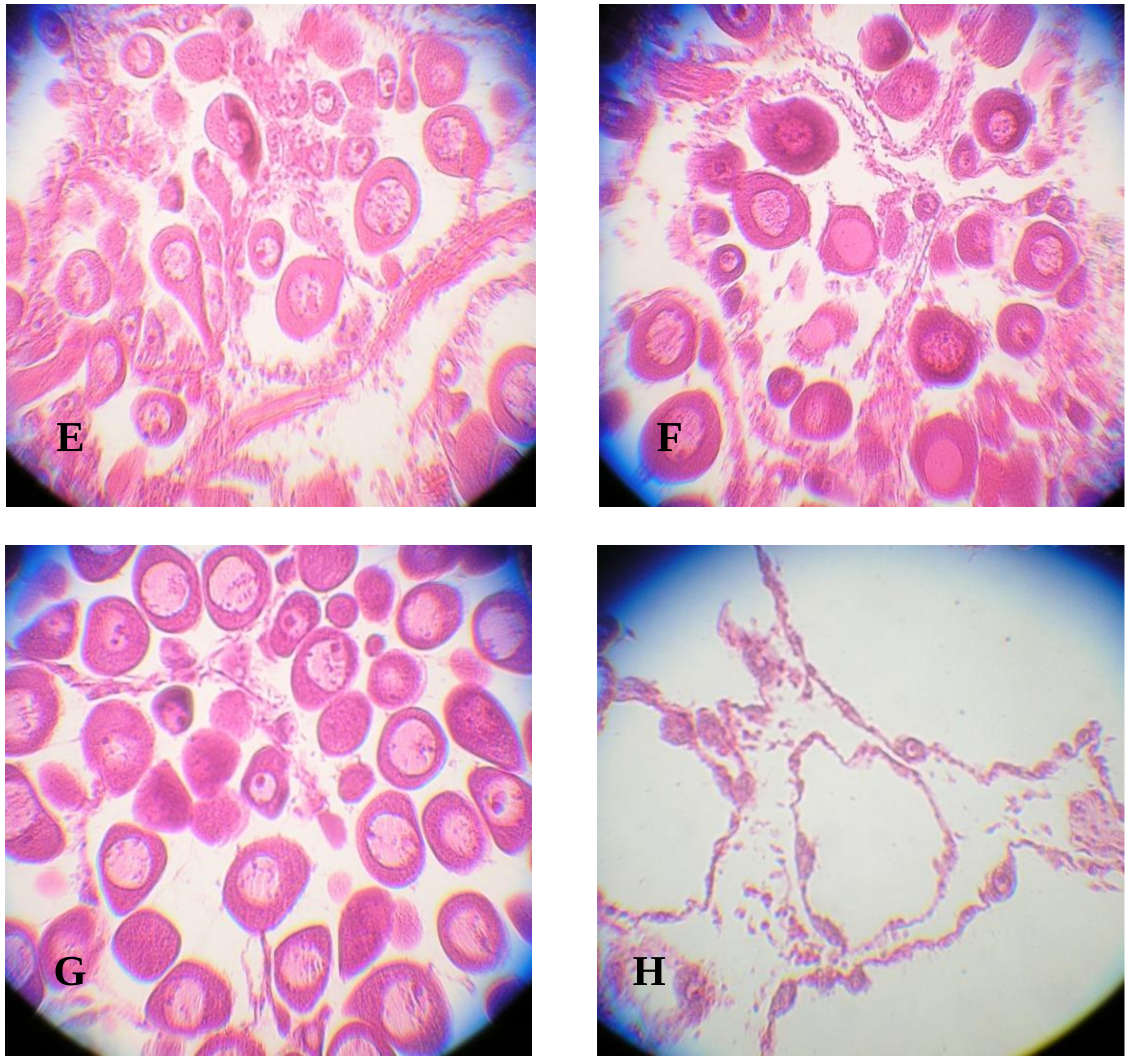


Figure 4.1B: Photographs of reproductive stages of female *Austrovenus stutchburyi.* Females (E) early active, (F) late active, (G) ripe, (H) spent. All photographs are viewed at x 40 magnification.

Age and Growth

The age of *Austrovenus stutchburyi* was determined by counting the annual shell growth rings (Figure 4.2) using the techniques described by Stephenson (1981) and Coutts (1974). Shells (n =10 for each site) from spring (2007) collected cockles were sectioned through the umbo to the ventral margin along the axis of maximum shell diameter, then the exposed edge of one half of the shell was ground. Using a stereomicroscope, the clearly seen dark bands (Figure 4.3) were counted.

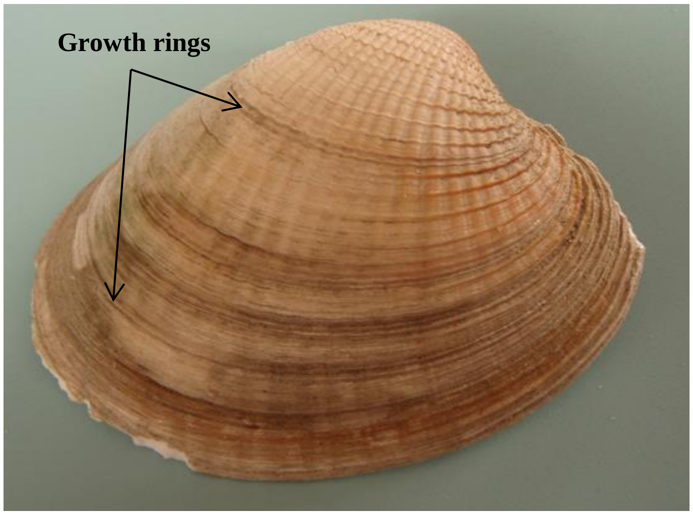


Figure 4.2: External view of *Austrovevnus stutchburyi* valve showing growth rings.

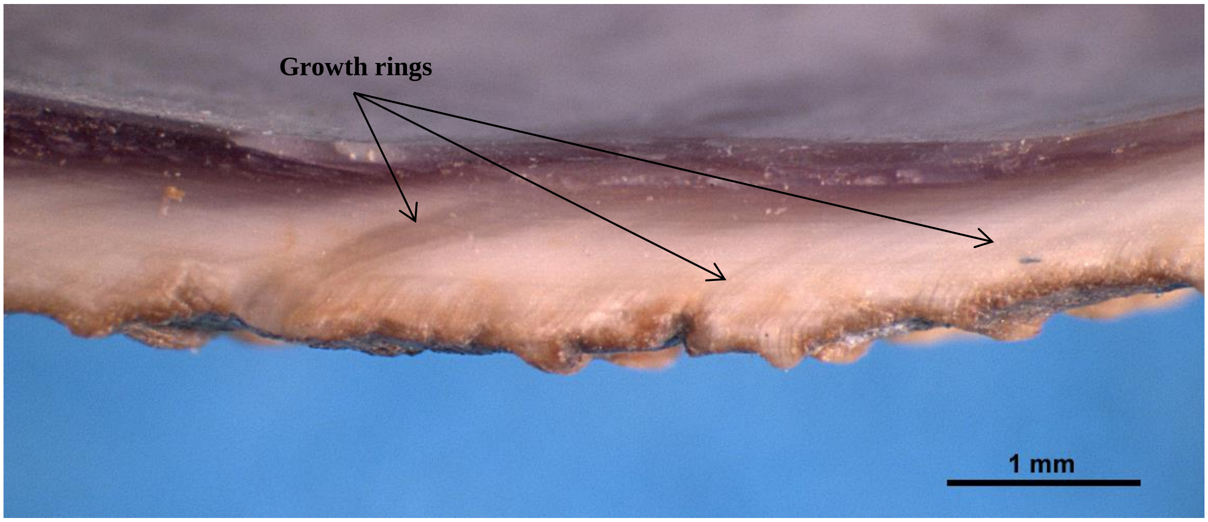


Figure 4.3: Sectioned *Austrovenus stutchburyi* valve showing growth rings.

Glycogen analysis

Three cockles, (*Austrovenus stutchburyi*) were collected seasonally from each of the eight sites for glycogen analysis. They were transported on ice to the laboratory and stored whole at -80°C until processing. The foot muscle was selected for glycogen analysis, leaving the gonad available for histological analysis of the reproductive stage.

Each cockle was removed from the freezer, weighed, measured and opened and the foot removed from the rest of the body which was transferred to Davison's fluid. Previous researchers (Stewart 2005) used 200mg of dried muscle tissue for analysis, but due to the small size of some of the cockles available for this study it was decided to work with wet tissue. Approximately 200mg of foot muscle from each cockle was thoroughly mixed for 15 minutes with 3ml 30% KOH in 20ml centrifuge tubes using a vortex mixer. The tubes were then heated for 60 minutes in a 50°C water bath, after which they were cooled in an ice bath. The volume in the tubes was then adjusted to 10mls using nanopure water. Triplicate 50µl amounts of each extract were mixed with 3ml of 0.15% anthrone in 82.4% sulphuric acid and heated for 10 minutes at 90°C in a water bath. They were then cooled in an ice bath and after 20-40 minutes the extinction was determined at 620nm using an Amershem Biosciences-Novaspec III–visible spectrometer. Blank samples (nanopure water) and standards (1 and 2 mg glucose/L) were also run in triplicate. Glycogen content (µg) is expressed as a percentage of wet flesh (foot) weight (g).

Condition analysis

Cockles for condition analysis were collected seasonally and annually at the time of the population surveys (*see* Chapter 3). Ten animals of a representative length range from each site were transported chilled to the laboratory where they were measured for length, width and height, and weighed prior to the separated shells and flesh being dried at 65°C for 72 hours, after which time, the shells and flesh were again weighed. Before opening all cockles were examined for the presence of epibionts, or evidence of predation. After opening, visual inspection of the flesh assessed general condition and whether or not gonad tissue was present, although the degree of gonad development was not recorded. Condition index was calculated for each cockle using the CI gravimetric formula of Crosby & Gale (1990).

Data analysis

Data were analysed for site-specific and between site differences using general linear models (ANOVA, ANCOVA, and chi-square) using STATISTICA© 6. Data were not transformed. The relationship between glycogen levels, condition indices, gonad indices and growth and environmental variables (salinity, nutrients, trace elements,, sediment and temperature) were tested using Spearman Rank correlation analysis ($p = 0.5$).

Results

Reproduction, age and growth

Reproductive stages

Histological analysis of gonad tissue allowed the cockles to be assigned to one of six pre-determined categories adapted from the method used by Stewart (2005). Very few samples were unable to be assigned a gonad index. No parasitic infestation of the foot muscle was noted in any of the slides.

Development stages were different for male and female cockles (Figure 4.4 A-D) with males showing reproductive activity over most of the year i.e. ripe or late active individuals present at all the sites at all seasons. Females showed most reproductive activity over the summer, and only limited activity during the other 3 seasons with late active individuals present in the spring at the Pa, Takamatua, and Barry's Bay sites. Mean cockle gonad index values (Figure 4.5A-D) illustrate this increase in gonad activity over the summer season.

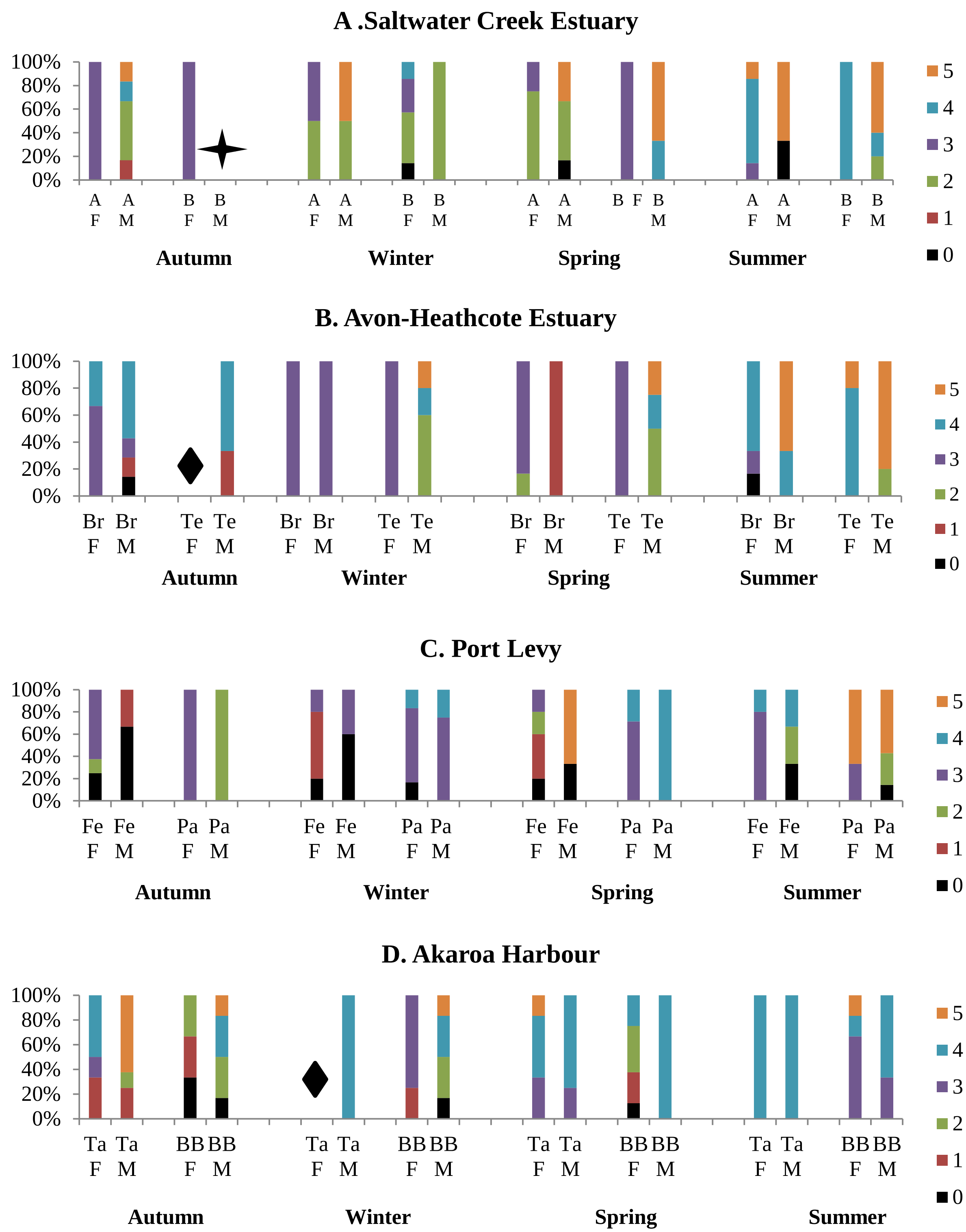


Figure 4.4A-D: Seasonal percentage development stages for the eight sites. Abbreviations: A=Saltwater A;B=Saltwater B;Br=Bromley;Te=Tern St; Fe= Fernlea; Pa=Pa; BB=Barry's Bay; Ta =Takamatua. Development stages: 0= indeterminate; 1= spent; 2= spawning; 3= early active ;4= late active; 5= ripe. F =female:M= male. Low salinity sites are the first in each pairing i.e. Saltwater A, Bromley, Fernlea and Takamatua). Star = no males; diamond = no females in sample.

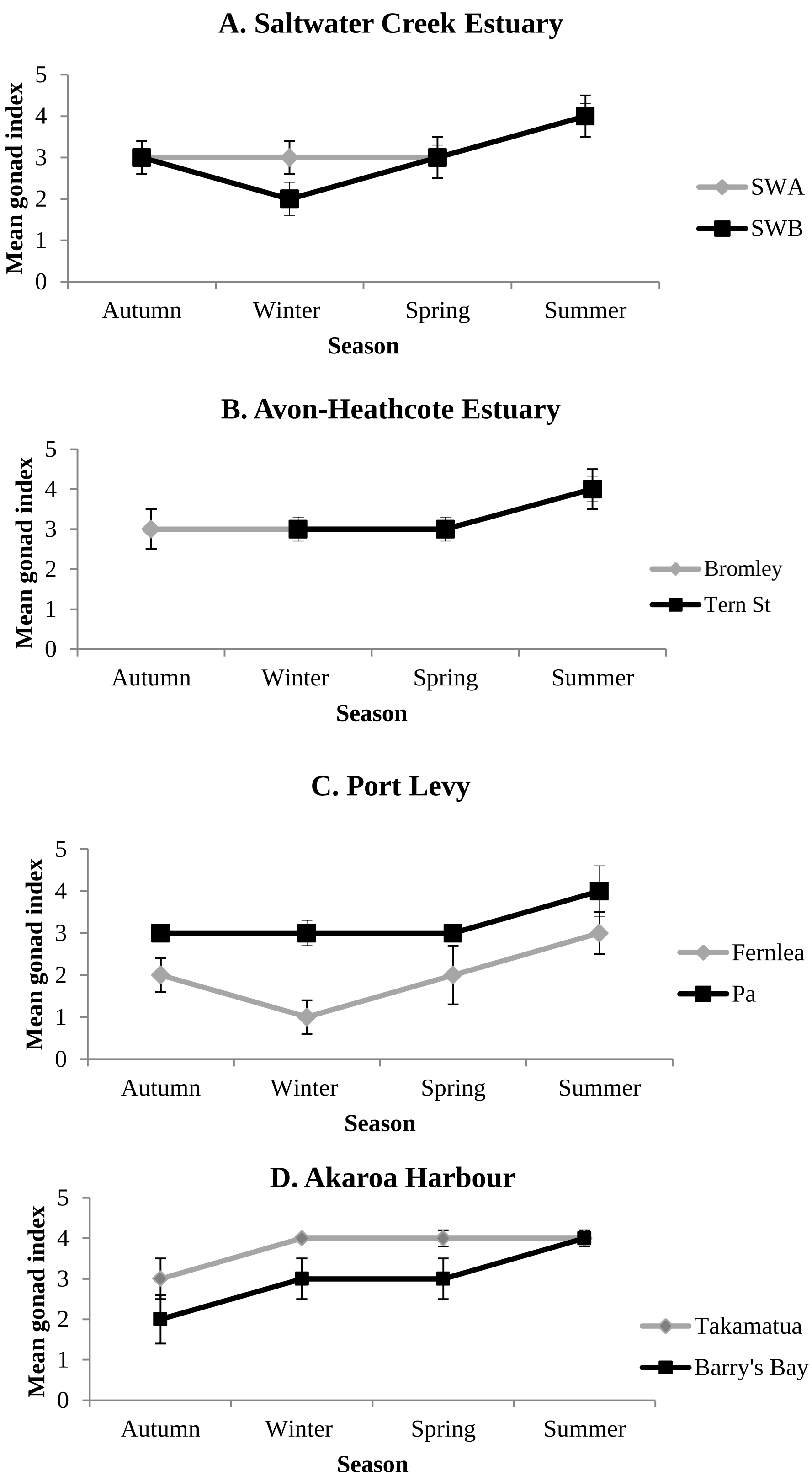


Figure 4.5 A-D: Mean seasonal cockle gonad index (±1SE) for the eight Canterbury estuarine systems. Dark lines indicate high salinity sites.

At Saltwater Creek all the females taken from both sites at the Autumn sampling were in the early active phase, while the males from the low salinity site A ranged from late active and ripe through to spawning and spent. No males were collected from site B at this time. Winter sampling showed females spawning and in the early active phase at Site A, while at the higher salinity site B there were late active and spent individuals along with these two phases. Approximately equal numbers of males were in the ripe or spawning phases at Site A in winter while all were spawning at Site B. Spring sampling at Site A showed a predominance of spawned females along with others in the early active phase. Males here had a few indeterminate indiviuals as well as ripe and spawning ones. At site B all the females collected were in the early active phase while the males were either late active or ripe. Summer sampling at both sites showed a peak in numbers for both males and females in the late active and ripe phases. There were some spawning males at site B.

Autumn cockle sampling at the Avon-Heathcote/Ihutai Estuary showed early and late active females at the low salinity Bromley site. The males here were mainly in the same phases, although there were some spent and indeterminate individuals also. For some unexplained reason there is no sample of cockles from Tern St at this time. At the winter sampling all the females at both sites and the males at Bromley were in the early active phase. At Tern St most of the males were spawning with the rest in the late active or ripe phases. Spring showed a similar situation with the females, although there were a few spawning individuals present at the Bromley site. Males at the high salinity Tern St site showed the same development stages as were present over winter, but at Bromley all individuals collected were spent. Again, the summer sampling at both sites showed a peak in the numbers of males and females in the late active and ripe phases.

Both sites at Port Levy/Koukourārata had female cockles in the early active phase at the autumn sampling time, with cockles from Fernlea, the low salinity site, also having some indeterminate and spawning individuals. Males at Fernlea were either indeterminate or spent, with those at the Pa site all spawning.Winter sampling at Fernlea showed indeterminate, spent and early active females, and indeterminate and early active males. At the Pa site there were indeterminate, early and late active females and early and late active males. A wider range of development stages was present at Fernlea during the spring sampling with indeterminate, spent, spawning and early active females, and indeterminate and ripe males. The Pa site had early and late active females and late active males. Females in the early and late active phases were present during the summer sampling at Fernlea while at the Pa site the females were either early active or ripe. Indeterminate, spawning and late active males were seen at Fernlea, with indeterminate, spawning and ripe individuals at the Pa.

The late active phase of cockle development predominated throughout the year at both Akaroa sites. At the autumn sampling, females in this stage, along with early active and spent ones were present at the low salinity site at Takamatua. Barry’s Bay showed indeterminate, spent and spawning females while the males were indeterminate, spawning, late active and ripe. At Takamatua the males were mainly ripe with some spawning and spent individuals.   At the winter sampling, no females were collected from Takamatua, and all the males were in the late active phase. At Barry’s Bay, the females were either spent or early active, and the males indeterminate, spawning, late active or ripe. Early and late active phases were seen in both males and females in the spring at Takamatua, as well as some ripe females. At Barry’s Bay the females fell into the indeterminate, spent, spawning and late active phases with the males being all late active. Summer sampling at Takamatua found all males and females in the late active phase, while at Barry's Bay there were some ripe females along with both early and late active males and females.

Based on these results, it appears that while the most reproductive activity occurs in the summer it is not limited to these warmer months. Males were active year round at all sites except Bromley (Avon-Heathcote/Ihutai) where activity was seen only in summer and autumn.

The gonad index for males and females at the low and high salinity sites for the four areas showed no effects for differing salinities (Table 4.3). There were, however, significant differences with season at Saltwater Creek (p < 0.001), Avon-Heathcote/Ihutai Estuary (*p* <0.001) and Akaroa Harbour (*p*<0.001). Interaction effects were apparent at Saltwater Creek (*p*=0.019), Port Levy/Koukourārata (*p*= 0.01) and Akaroa Harbour (p=0.002).

Table 4.3: Results of repeated measures ANOVA comparing gonad index of *Austrovenus stutchburyi* at high and low salinity sites at each of the 4 estuaries. Significant values are shown in bold.

Area	*Source*	Males *df*	Males F value	Males *p value*	Females *df*	Females F value	Females *p value*
Saltwater Creek	**Site**	1	1.48	0.225	1	0.13	0.725
	Season	3	2.23	0.134	3	14.90	**<0.001**
	Site x Season	3	6.36	**0.002**	3	3.85	**0.019**
Avon-Heathcote	**Site**	1	1.41	0.262	1	0.44	0.518
	Season	3	3.01	0.046	3	11.33	**<0.001**
	Ssite x Season	3	5.98	**0.003**	3	2.80	0.053
Port Levy	**Site**	1	0.79	0.395	1	4.14	0.063
	Season	3	30.31	0.033	3	1.42	0.251
	Site x Season	3	2.40	0.089	3	4.32	**0.01**
Akaroa Harbour	**Site**	1	0.76	0.402	1	4.88	0.046
	Season	3	1.43	0.253	3	8.54	**<0.001**
	Site x Season	3	4.83	**0.007**	3	5.78	**0.002**

Mean (males and females combined) gonad index (Figure 4.5A-D) for all sites peaked over summer, with the lowest values occurring at autumn. The gonad index for cockles at both sites at Saltwater Creek and the Avon- Heathcote/Ihutai Estuary were similar except for Site B at Saltwater Creek at the winter season and Tern St (Avon-Heathcote/Ihutai) in the autumn which both had lower values (Figure 4.5.A & B). At Port Levy/Koukourārata the lower salinity Fernlea site consistently had a lower cockle gonad index (Figure 4.5.C). The opposite trend was seen at Akaroa Harbour where the high salinity site had lower gonad indices (Figure 4.5.D).

Oocyte number and size

Table 4.4: Results of repeated measures ANOVA comparing oocyte number of *Austrovenus stutchburyi* at all sites. Significant values are shown in bold.

Source	*df*	*F value*	*P-value*
Season	3	1.15	0.347
Site	7	8.65	**<0.001**
Season x Site	21	7.45	**<0.001**

Table 4.5: Results of repeated measures ANOVAs comparing oocyte number of *Austrovenus stutchburyi* at high and low salinity sites at each of the 4 estuaries. Significant values are shown in bold.

Area	Source	*df*	*F value*	*P-value*
Saltwater Creek	Season	3	8.37	**<0.001**
	Site	1	3.57	0.070
	Season x Site	3	5.22	**0.006**
Avon-Heathcote	Season	3	4.81	**0.008**
	Site	1	6.94	**0.014**
	Season x Site	3	2.47	0.084
Port Levy	Season	3	0.88	0.463
	Site	1	0.86	0.363
	Season x Site	3	2.22	0.109
Akaroa Harbour	Season	3	6.37	**0.002**
	Site	1	3.17	0.086
	Season x Site	3	5.84	**0.003**

Oocyte numbers in female *Austrovenus stutchburyi* determined seasonally varied between sites (Table 4.4). There was no significant variation between season, but there was an interaction between site and season. Significant differences in oocyte numbers between high and low salinity sites of the four areas over the 4 seasons were apparent only in the Avon-Heathcote/Ihutai Estuary (Table 4.5, p <0.014) where there was also a seasonal variation (p=

0.008). Cockles from Port Levy/Koukourārata did not show any significant differences in oocycte numbers , whereas Saltwater Creek and Akaroa Harbour had significant seasonal effects (SWC *p*=<0.000; Akaroa *p*=0.002) and also interactions between season and salinity (SWC *p*=0.006; Akaroa *p*=0.003).

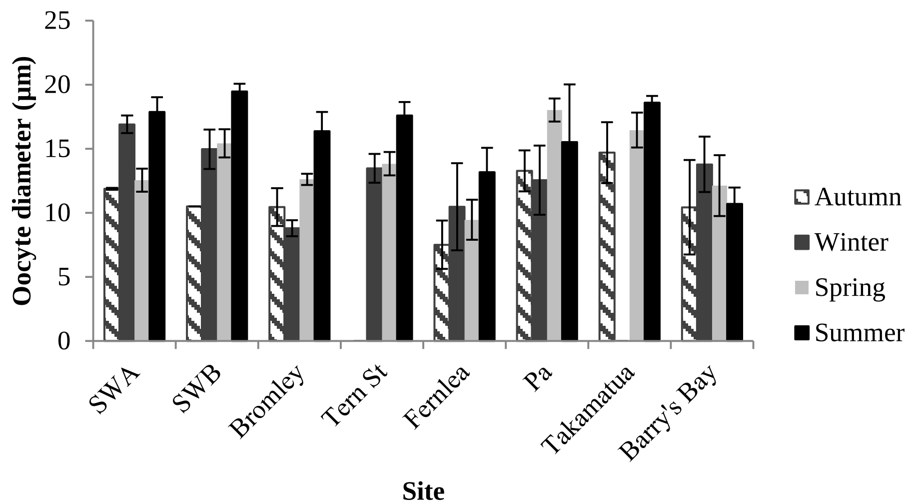


Figure 4.6 :Mean seasonal oocyte diameter (±1SE) for female *Austrovenus stutchburyi* collected from the eight Canterbury sites.

There were no significant differences in oocyte diameter between the sites irrespective of salinity (Tables 4.6, & 4.7) but there was a seasonal difference, and a site x season interaction with all the sites combined (Table 4.6. Repeated measures ANOVA). However, while cockles from the Port Levy/Koukourārata sites did not show any significant differences in oocyte diameter between high and low salinity, Saltwater Creek and Akaroa Harbour cockles both showed seasonal (SWC *p*=<0.000; Akaroa *p*=0.009) and season x salinity interactions (SWC *p*=0.021; Akaroa *p*=0.005). Significant seasonal differences were detected at the Avon-Heathcote estuary (*p*= <0.000). The largest oocytes occurred during summer (Figure 4.6) except for Barry’s Bay (winter) and the Pa site at Port Levy (spring).

Table 4.6: Results of repeated measures ANOVA comparing oocyte diameter of *Austrovenus stutchburyi* at all sites. Significant values are shown in bold.

Source	*df*	*F value*	*P-value*
Season	3	2.96	**0.05**
Site	7	0.75	0.632
Season x Site	21	6.153	**<0.001**

Table 4.7. Results of repeated measures ANOVAs comparing oocyte diameter of *Austrovenus stutchburyi* at high and low salinity sites at each of the 4 estuaries. Significant values are shown in bold.

Area	**Source**	*df*	*F value*	*P-value*
Saltwater Creek	Season	3	9.67	**<0.001**
	Site	1	2.07	0.161
	Season x Site	3	3.82	**0.021**
Avon-Heathcote	Season	3	8.68	**<0.001**
	Site	1	0.88	0.357
	Season x Site	3	2.12	0.122
Port Levy	Season	3	0.09	0.962
	Site	1	1.06	0.313
	Season x Site	3	2.19	0.113
Akaroa Harbour	Season	3	4.70	**0.009**
	Site	1	0.04	0.846
	Season x Site	3	5.37	**0.005**

There were significant positive correlations between site and mean gravimetric condition index (0.908); number of ova and gonad index (0.783) and ova diameter (0.872); (Table 4.8). Mean CI gravimetric was positively correlated with salinity (0.704) and TVS with the female:male ratio (0.808) while mean cockle length and water temperature were also positively correlated (0.896).

Table 4.8: Results of Spearman rank correlation analysis investigating the relationships between site, cockle shell length, gonad index, mean number of ova per follicle, environmental and population variables and mean ova diameter in female *Austrovenus stutchburyi*. Marked correlations (bold font) are significant at $p < 0.05$.

	Site	**Mean length**	**Density**	**% silt**	**% sand**	**Salinity**	**Mean CI grav**	**Temp**	**TVS**	**Gonad index**	**Mean MPI**	**F:M ratio**	**Mean glycogen**	**Oocyte diam**	**Oocyle number**
Site	1														
Mean length	-0.275	1													
Density	0.239	-0.591	1												
% silt	-0.159	-0.309	-0.302	1											
% sand	0.159	0.309	0.302	**-1**	1										
Salinity	0.59	0.126	0.024	-0.465	0.465	1									
Mean CI grav	**0.908**	-0.375	0.499	-0.279	0.27	**0.704**	1								
Temp	0.007	**0.896**	-0.332	-0.506	0.506	0.189	-0.075	1							
TVS	-0.622	-0.224	-0.107	0.159	-0.159	-0.552	-0.585	-0.346	1						
Gonad index	0.164	-0.373	0.19	-0.165	0.165	0.129	0.09	-0.482	0.058	1					
Mean MPI	**0.731**	-0.018	0.012	-0.156	0.516	0.597	0.67	0.285	-0.315	-0.039	1				
F:M ratio	-0.282	-0.092	-0.268	-0.078	0.078	-0.34	-0.353	-0.057	**0.808**	-0.076	0.244	1			
Mean Glycogen	0.542	-0.556	0.052	-0.009	0.009	0.468	0.498	-0.553	0.091	0.649	0.427	0.179	1		
Oocyte diam	-0.424	0.073	-0.334	0.044	-0.044	-0.075	-0.459	-0.224	0.635	0.495	-0.362	0.356	0.373	1	
Oocyte number	-0.029	-0.18	-0.129	0.019	-0.019	0.002	-0.146	-0.369	0.422	**0.783**	-0.169	0.221	0.639	**0.872**	1

Male gonad follicle number and size

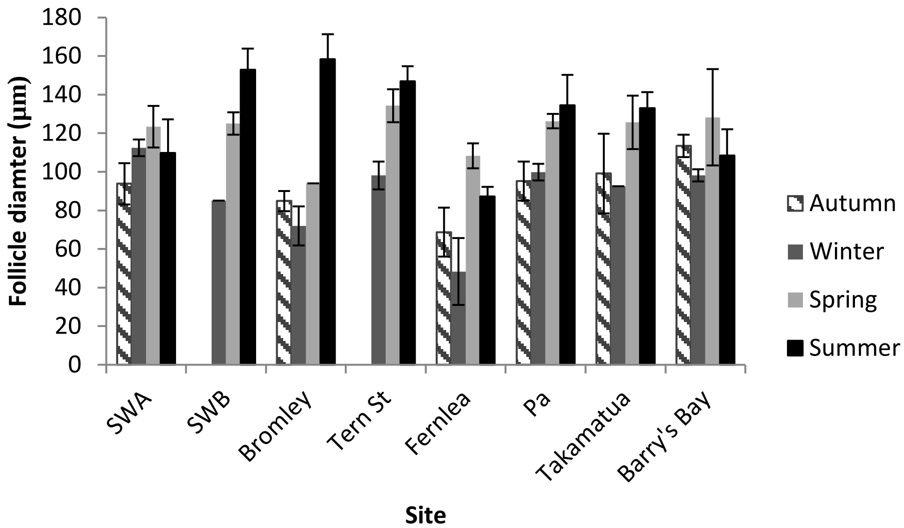


Figure 4.7 : Mean seasonal follicle diameter (±1SE) for male *Austrovenus stutchburyi* collected from the eight Canterbury sites.

Generally there was an increase in the diameter of the follicles in male *Austovenus stutchburyi* over the spring/summer periods with the peak occurring during in summer except at the low salinity Saltwater Creek A and Fernlea sites, and the high salinity Barry's Bay site (Figure 4.7).

Table 4.9: Results of repeated measures ANOVA comparing male gonad follicle number for *Austrovenus stutchburyi* at all sites. Significant values are shown in bold.

Source	*df*	*F value*	*P-value*
Season	3	0.13	0.944
Site	7	4.32	**<0.001**
Season x Site	21	5.91	**<0.001**

Table 4.10. Results of repeated measures ANOVAs comparing male gonad follicle numbers for *Austrovenus stutchburyi* at high and low salinity sites at each of the 4 estuaries. Significant values are shown in bold.

Area	Source	*df*	*F value*	*P-value*
Saltwater Creek	Season	3	3.04	**0.047**
	Site	1	9.13	**0.006**
	Season x Site	3	2.99	**0.050**
Avon-Heathcote	Season	3	3.34	**0.035**
	Site	1	0.88	0.357
	Season x Site	3	21.63	**<0.001**
Port Levy	Season	3	0.17	0.914
	Site	1	5.05	**0.033**
	Season x Site	3	0.71	0556
Akaroa Harbour	Season	3	1.75	0.182
	Site	1	2.16	0.154
	Season x Site	3	4.19	**0.015**

Table 4.11: Results of repeated measures ANOVA comparing male gonad follicle diameter of *Austrovenus stutchburyi* at all sites. Significant values are shown in bold.

Source	*df*	*F value*	*P-value*
Season	3	0.21	0.886
Site	7	4.35	**<0.001**
Season x Site	21	6.02	**<0.001**

Table 4.12. Results of repeated measures ANOVAs comparing male gonad follicle diameter of *Austrovenus tutchburyi* at high and low salinity sites at each of the 4 estuaries. Significant values are shown in bold.

Area	Source	*df*	*F value*	*P-value*
Saltwater Creek	Season	3	3.19	**0.040**
	Site	1	8.98	**0.006**
	Season x Site	3	3.06	**0.046**
Avon-Heathcote	Season	3	2.77	0.062
	Site	1	1.25	0.274
	Season x Site	3	20.54	**<0.001**
Port Levy	Season	3	0.36	0.780
	Site	1	5.11	0.032
	Season x Site	3	0.96	0.426
Akaroa Harbour	Season	3	2.51	0.080
	Site	1	1.79	0.193
	Season x Site	3	4.90	**0.008**

Follicle numbers in male *Austrovenus stutchburyi* determined seasonally varied between sites (Table 4.9). There was no significant variation between season, but there was an interaction between site and season($p<0.001$). Significant differences in follicle numbers between high and low salinity sites (Table 4.10) of the four areas over the 4 seasons were identified at the Saltwater Creek estuary with season (p =0.047), site (p =0.006) and season x site interaction (p =0.05). cockles from the Avon-Heathcote/Ihutai Estuary (Table 4.10) showed a season variation (p = 0.035) and a season x site interaction ($p<0.001$). Cockles from Port Levy/Koukourārata showed significant difference (p =0.033) for site only, while those from Akaroa Harbour showed a season x site interaction (p =0.015).

There were significant differences in male follicle diameter similar to the pattern shown with follicle numbers i.e. there was no significant variation between season, but there was for site with an interaction between site and season ($p<0.001$) (Table 4.11). Follicle diameter differed significantly between high and low salinity sites in the four estuarine areas (Table 4.12). Male follicle diameter in cockles from Saltwater Creek showed significant differences with season (p =0.04), site (p =0.006) and season x site interaction (p =0.046). Again, like follicle numbers, male follicle diameter in cockles from Port Levy did not show any significant differences, with Avon-Heathcote/Ihutai Estuary and Akaroa Harbour both showing season x site interactions (Avon-Heathcote p=0.001; Akaroa p= 0.008). The largest male follicles occurred during summer (Figure 4.7) except for Saltwater Creek A, Fernlea and Barry's Bay where the larger follicles occurred in the spring.

There were significant positive correlations (Table 4.13) between site and gravimetric condition index (p =0.908) and MPI (p =0.732); mean size and temperature (p =0.897); salinity and CI gravimetric (p =0.704); gonad index and follicle number (p = 0.735) and TVS and female:male ratio (p =0.808).

Table 4.13: Results of Spearman rank correlation analysis investigating the relationships between site, cockle shell length, gonad index,mean number of follicles, environmental and population variables and mean follicle diameter in male *Ausrovenus stutchburyi* at 8 canterbury sites. Marked correlations (bold font) are significant at *p*< 0.05.

	Site	Mean length	Density	% silt	% sand	Salinity	Mean CI grav	Temp	TVS	Gonad index	Mean MPI	F:M ratio	Mean glycogen	Follicle number	Follicle diam
Site	1														
Mean length	-0.099	1													
Density	0.239	-0.149	1												
% silt	-0.159	-0.669	-0.302	1											
% sand	0.159	0.669	0.302	**-1**	1										
Salinity	0.59	0.126	0.024	-0.468	0.465	1									
Mean CI grav	**0.908**	-0.124	0.499	-0.279	0.279	**0.704**	1								
Temp	0.007	**0.897**	-0.332	-0.506	0.506	0.189	-0.075	1							
TVS	-0.622	-0.067	-0.11	0.159	-0.159	-0.552	-0.585	-0.349	1						
Gonad index	-0.069	-0.182	0.268	-0479	0.479	0.404	0.117	-0.389	0.189	1					
Mean MPI	**0.732**	0.05	0.099	-0.437	0.437	0.535	0.694	0.153	-0.299	0.143	1				
F:M ratio	-0.282	0.107	-0.268	-0.078	0.078	-0.34	-0.353	-0.057	**0.808**	0.113	0.247	1			
Mean Glycogen	0.542	-0.427	0.052	-0.009	0.009	0.468	0.498	-0.553	0.091	0.529	0.435	0.179	1		
Follicle number	-0.536	0.261	-0.192	-0.443	0.443	0.156	-0.419	0.007	0.494	**0.735**	-0.218	0.353	0.169	1	
Follicle diam	-0.029	-0.067	-0.129	0.019	0.019	0.002	-0.146	-0.369	0.422	0.416	-0.242	0.221	0.639	0.504	1

Sex ratio

The sex ratio at all sites over all seasons for cockles selected for development analysis (Table 4.14) was close to equal (1♀:1♂) for half (16/32) the samples (x^2 =9.92, df=7; p=0.05), as it was also within the 4 areas when comparing high and low salinity sites (x^2 =1.8 or less, df=4, p=0.05). At site B at the Saltwater Creek estuary (winter) Bromley (spring) and Pa (spring) there was a bias towards females (x^2=6.05).

Table 4.14: Sex ratios (♀:♂) for cockles processed for development analysis. Shaded cells identify high salinity sites. (n=10)

Site / **Season**	**Saltwater A**	**Saltwater B**	**Bromley**	**Tern St**	**Fernlea**	**Pa**	**Takamatua**	**Barry's Bay**
Autumn	3:7	All female	4:6	missing	7:1	6:4	6:4	3:7
Winter	4:6	9:1	5:5	5:5	5:5	5:5	All male	4:6
Spring	4:6	7:3	9:1	6:4	5:5	8:2	6:4	6:4
Summer	7:3	5:5	7:3	5:5	7:3	3:7	4:6	7:3

Age and growth

The range of shell lengths for the cockles available for age analysis was lower at low salinity sites than at the high salinity ones (Table 4.15). However, there was not a similar pattern with age based on growth rings (Table 4.15) with Saltwater Creek A (2-6 rings) and Takamatua (2-7 rings) (Akaroa Harbour), both low salinity sites, having a similar age range to the cockles from Tern Street in the the Avon-Heathcote/Ihutai Estuary (2-7 rings) and the Pa site at Port Levy/Koukourārata (2-7 rings), two of the higher salinity sites.

Table 4.15: Shell length and growth ring ranges for cockles from paired low and high salinity sites in the Canterbury region. (n=20).

Area	Shell length range (mm)		Shell ring range	
	Lower salinity site	Higher salinity site	Lower salinity site	Higher salinity site
Saltwater Creek	19.25-33.73	20.77-36.09	2-6	1-3
Avon-Heathcote	20.47-38.63	25.08-47.73	2-4	2-7
Port Levy	20.05-35.12	23.89-47.67	1-4	2-7
Akaroa Harbour	17.07-23.3	17.91-25.75	2-7	1-3.5

Table 4.16: ANCOVA describing the relationship between shell ring number at high and low salinity sites in the Canterbury region.

Source of Variation	*df*	*MS*	*F*	*P-value*
Saltwater Creek	1,37	40.04	35.07	**<0.001**
Avon-Heathcote	1,37	5.44	6.93	**0.012**
Port Levy	1,37	5.37	7.41	**0.009**
Akaroa Harbour	1,37	5.59	4.22	**0.047**

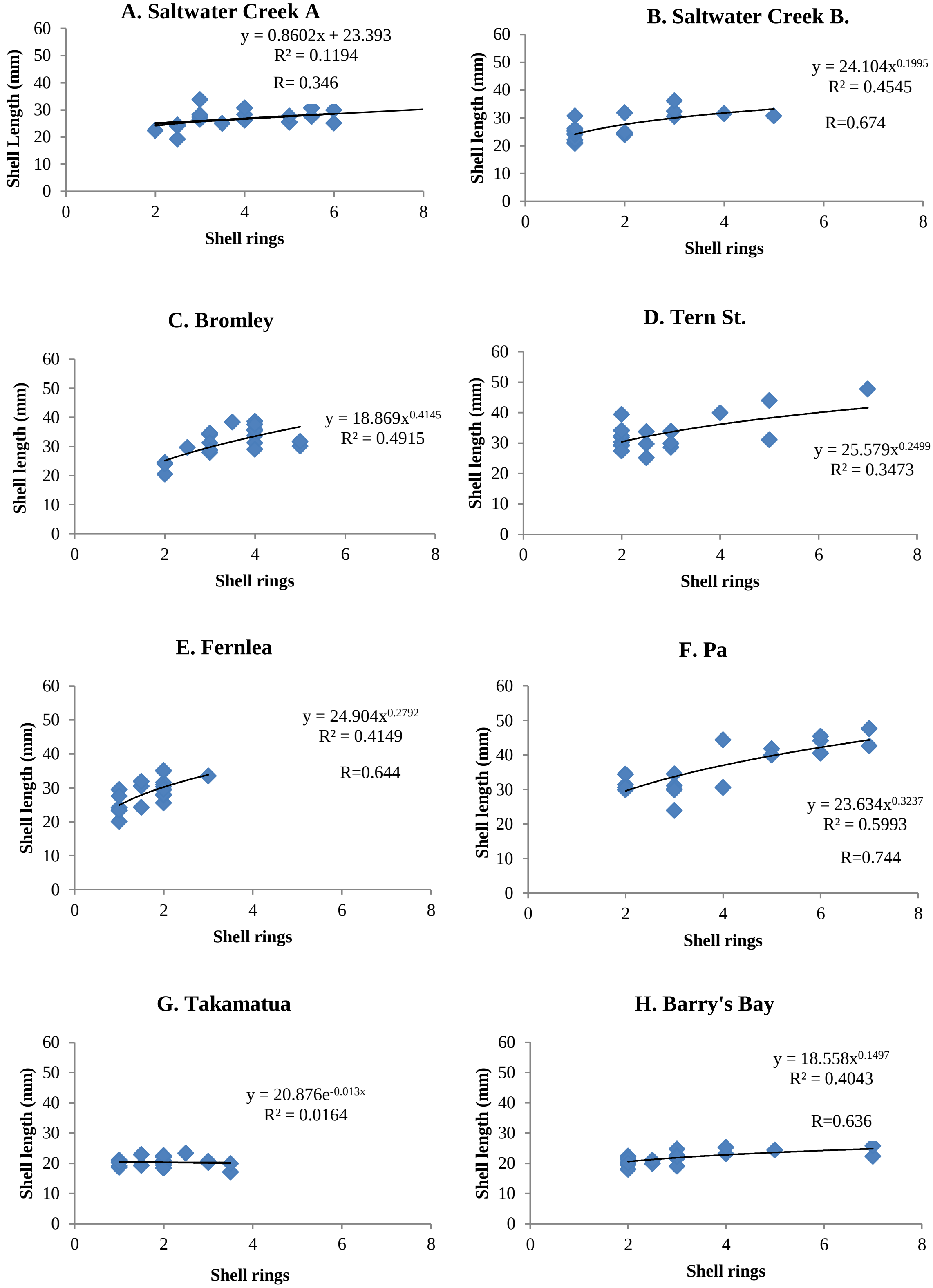


Figure 4.8 A-H. The relationship between shell length and number of growth rings in *Austrovenus stutchburyi* at the 8 study sites. N=20 in all cases. Graphs B,C,D,E,F and H all show significant relationships between shell length and growth rings.

There were significant differences in the age of cockles based on shell ring numbers between the eight study sites (Figure 4.8). Significant relationships between shell length and number of shell growth rings were identified at six of the eight sites with larger cockles being older based on shell ring numbers. Four of these sites were high salinity sites. Saltwater B (R = 0.645) had a shell length range of 20-38mm, Tern St (R = 0.641) a range of 24-48mm, Pa (R =0.811) 23-50mm, and Barry's Bay (R=0.595) 18-30mm. The other two sites showing significant relationships between length and ring number were Bromley (R =0.626) with a shell length range of 20-40mm, and Fernlea (R =0.626) with a range of 20-35mm, both low salinity sites. A significant relationship was not apparent at Saltwater Creek A, while at Takamatua age was not related to size as these small cockles could have up to seven growth rings (Table 4.15). Histological examination of gonad tissues also supports the maturity of these small cockles with most having active gonad tissue.

Table 4.17: Mean shell length (mm) of cockles with 3 growth rings, and number of growth rings for shells 25mm in length from paired low and high salinity sites in the Canterbury region. (n=20 for all sites).

Area	***Shell length (mm)***		***Growth rings***	
	Lower salinity site	**Higher salinity site**	**Lower salinity site**	**Higher salinity site**
Saltwater Creek	21.5	35	3.5	2
Avon-Heathcote	30	32	2.5	2
Port Levy	33	30	1.5	1.5
Akaroa Harbour	20	22	3	3

Shell growth rate was similar at the low salinity sites at Avon-Heathcote/Ihutai (Bromley) and Port Levy/Koukourārata (Fernlea) and the high salinity sites at Saltwater Creek (Site B), Avon-Heathcote (Tern St) and Port Levy/Koukourārata (Pa) (Table 4.17) with cockles attaining a length of 30-35mm in the time that they laid down 3 growth rings. At the low salinity Saltwater Creek site (A) and at both Akaroa sites shell growth was much slower with cockles from these sites reaching only 20-22mm in length over the time taken to lay down 3 rings.

Glycogen

The glycogen levels in the foot muscle of *Austrovenus stutchburyi* determined seasonally varied between sites with Barry's Bay in Akaroa harbour having consistently higher values (0.35- 0.4% foot wet weight) than the other sites (0.1-0.3% foot wet weight) (Table 4.18; Figure 4.9). Although there was no significant variation between season, there was an interactive effect of site and season. Saltwater Creek A, Pa and Barry's Bay showed slight drops in glycogen levels at spring, while Tern Street and Fernlea showed similar reductions in winter. Significant differences in cockle glycogen levels between high and low salinity sites of the four areas over the 4 seasons were apparent only at Akaroa Harbour (Table 4.19, p <0.001), with interactive effects of site and season at Port Levy (p =0.026) and Akaroa Harbour (p = 0.032). There was a significant relationship (R= 0.458) between gonad index and glycogen (Figure 4.10) in *Austrovenus stutchburyi* from the Canterbury sites

The sex ratio observed in the cockles selected for glycogen analysis (n=3) (x^2=2.54, df=7; p=0.05) showed both males and females present, except for Bromley (summer) Fernlea (autumn and spring) and Takamatua (summer) where all three animals were males. In spring, the three animals at Tern St and Takamatua were female, as were the three at the Pa site in summer.

Table 4.18: Results of repeated measures ANOVA comparing glycogen levels of *Austrovenus stutchburyi* foot muscle at the 8 sites. Significant values are shown in bold.

Source	*df*	*MS*	*F value*	*P-value*
Season	3	0.017	1.99	0.195
Site	7	0.176	27.4	**<0.001**
Season x Site	21	0.012	1.89	**< 0.001**

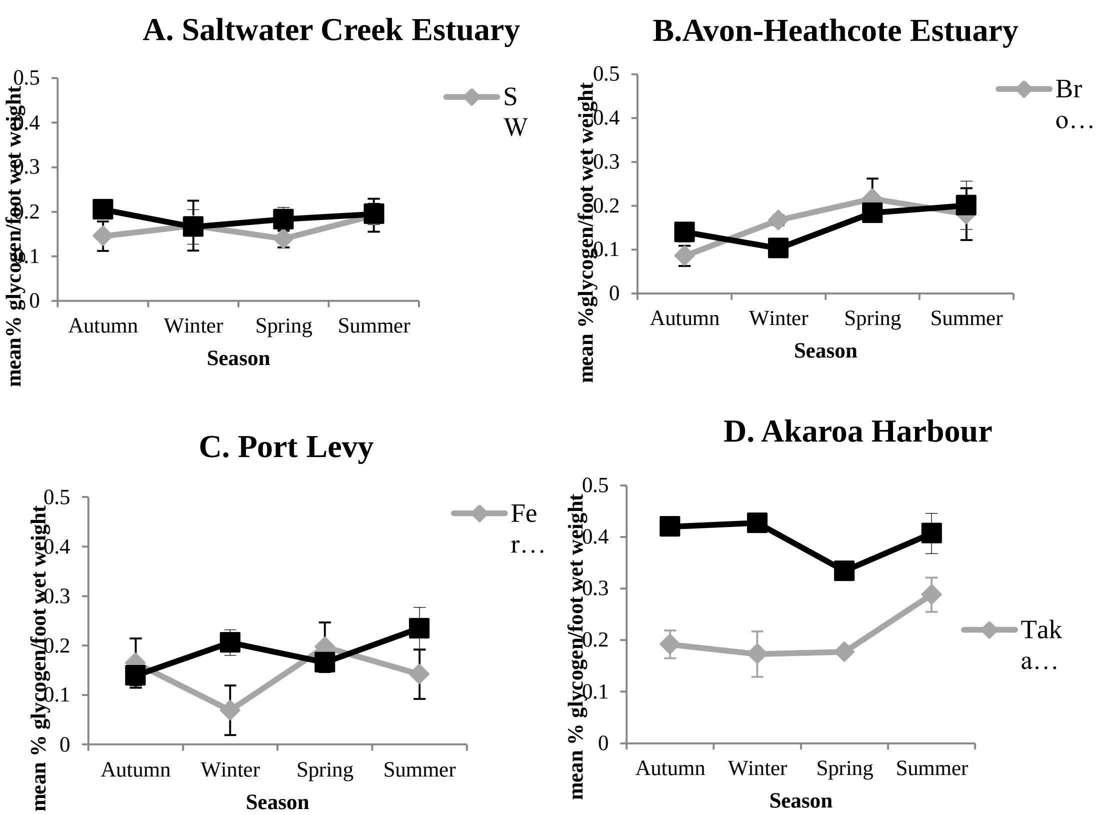


Figure 4.9:A-D. Seasonal mean glycogen content of *Austrovenus stutchburyii* presented as a percentage of wet foot weight (±1SE). *n*= 3 cockles per site. Dark lines indicate higher salinity sites.

Table 4.19: Results of repeated measures ANOVAs comparing glycogen levels in the foot muscle of *Austrovenus stutchburyi* at high and low salinity sites at each of the 4 estuaries over 4 seasons. Significant values are shown in bold.

Area	**Source**	***df***	***F value***	***P-value***
Saltwater Creek	Season	3	0.19	0.898
	Site	1	3.49	0.098
	Season x Site	3	0.61	0.629
Avon-Heathcote	Season	3	1.91	0.206
	Site	1	0.19	0.674
	Season x Site	3	2.09	0.180
Port Levy	Season	3	0.75	0.551
	Site	1	4.38	0.070
	Season x Site	3	5.35	**0.026**
Akaroa Harbour	Season	3	3.30	0.077
	Site	1	125.60	**<0.001**
	Season x Site	3	4.90	**0.032**

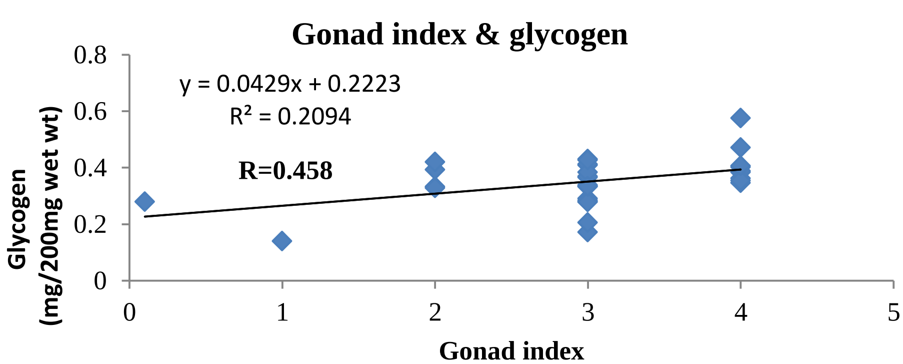


Figure 4.10: Mean seasonal values illustrating the relationship of gonad index and glycogen for *Austrovenus stutchburyi* from the 8 Canterbury sites. Significant values are in bold font.

Condition index

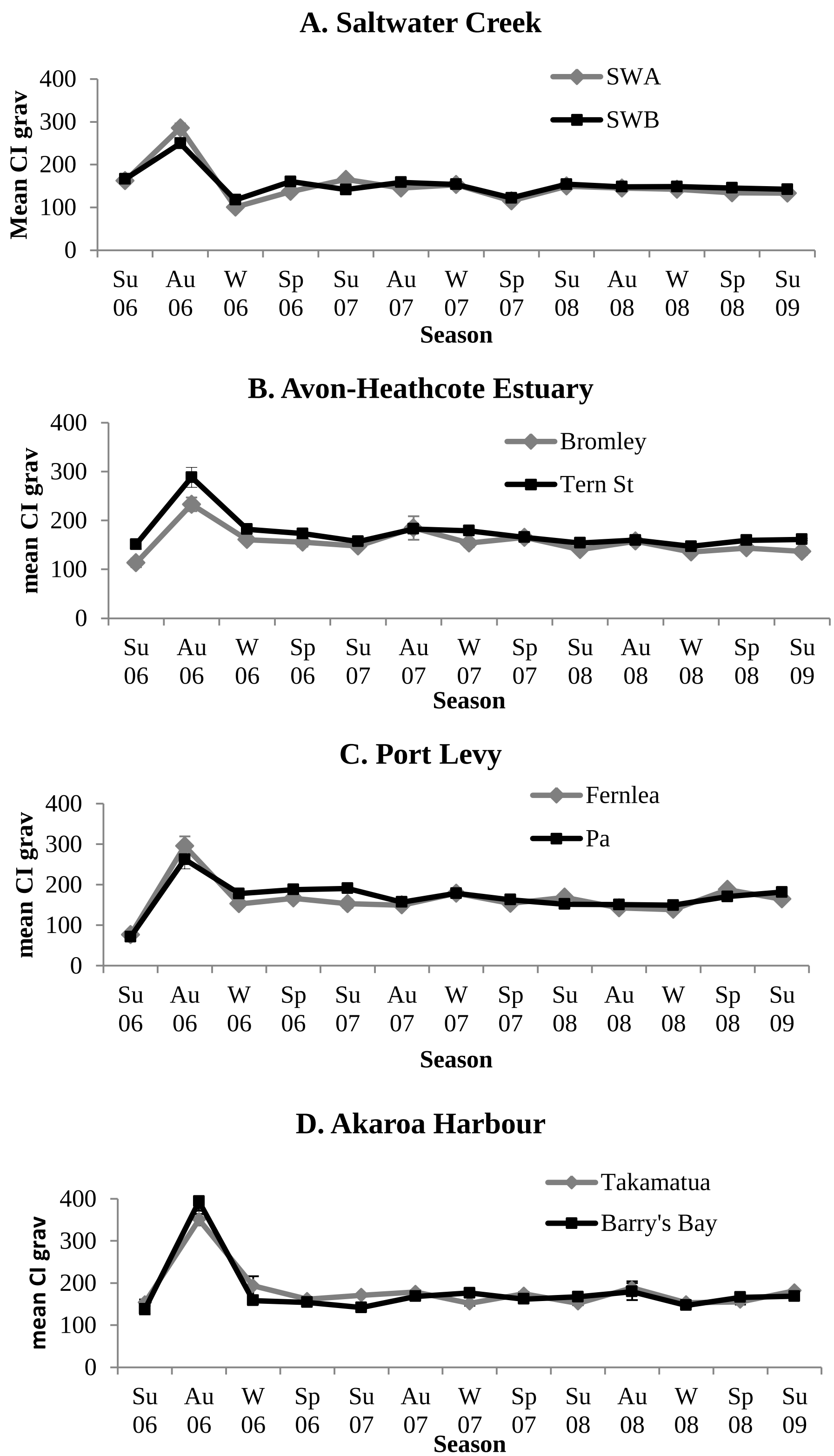


Figure 4.11: Mean condition index (gravimetric) (± 1SE) for *Austovenus stutchburyi* from 8 sites over 13 seasons. Dark lines represent high salinity sites.

The mean gravimetric condition index of *Austrovenus stutchburyi* determined seasonally and annually varied both between sites and seasons (Table 4.20: Figure 4.11), and between years within sites (Figure 4.12). Significant differences in cockle condition index between high and low salinity sites over the 13 seasons were apparent (Table 4.21, p <0.001). At Saltwater Creek there was a significant interaction effect showing along with temporal and spatial differences. At the Avon-Heathcote/Ihutai Estuary, there were salinity and seasonal differences, while both Port Levy/Koukourārata and Akaroa Harbour showed seasonal differences as well as site x season interactions. Over the seven summers a similar pattern (Table 4.22) was apparent although a salinity effect showed at both the Avon-Heathcote/Ihutai and Port Levy/Koukourārata (Table 4.23; Figure 4.12).

Plots of the mean seasonal conditions indices show a distinct peak occurring at the autumn 2006 sampling time (Figure 4.11). Apart from this, the indices did not show such large fluctuations for high and low salinity sites over the other seasons. Graphs of the mean condition indices (Figure 4.11) over the seven summer sampling periods illustrate the differences in condition index between the low and high salinity sites.

Less than 5% of the cockles assessed for condition index showed evidence of epibionts or predation, and all had some degree of gonad tissue visible. Parasitic infestation was not detected in any cockles.

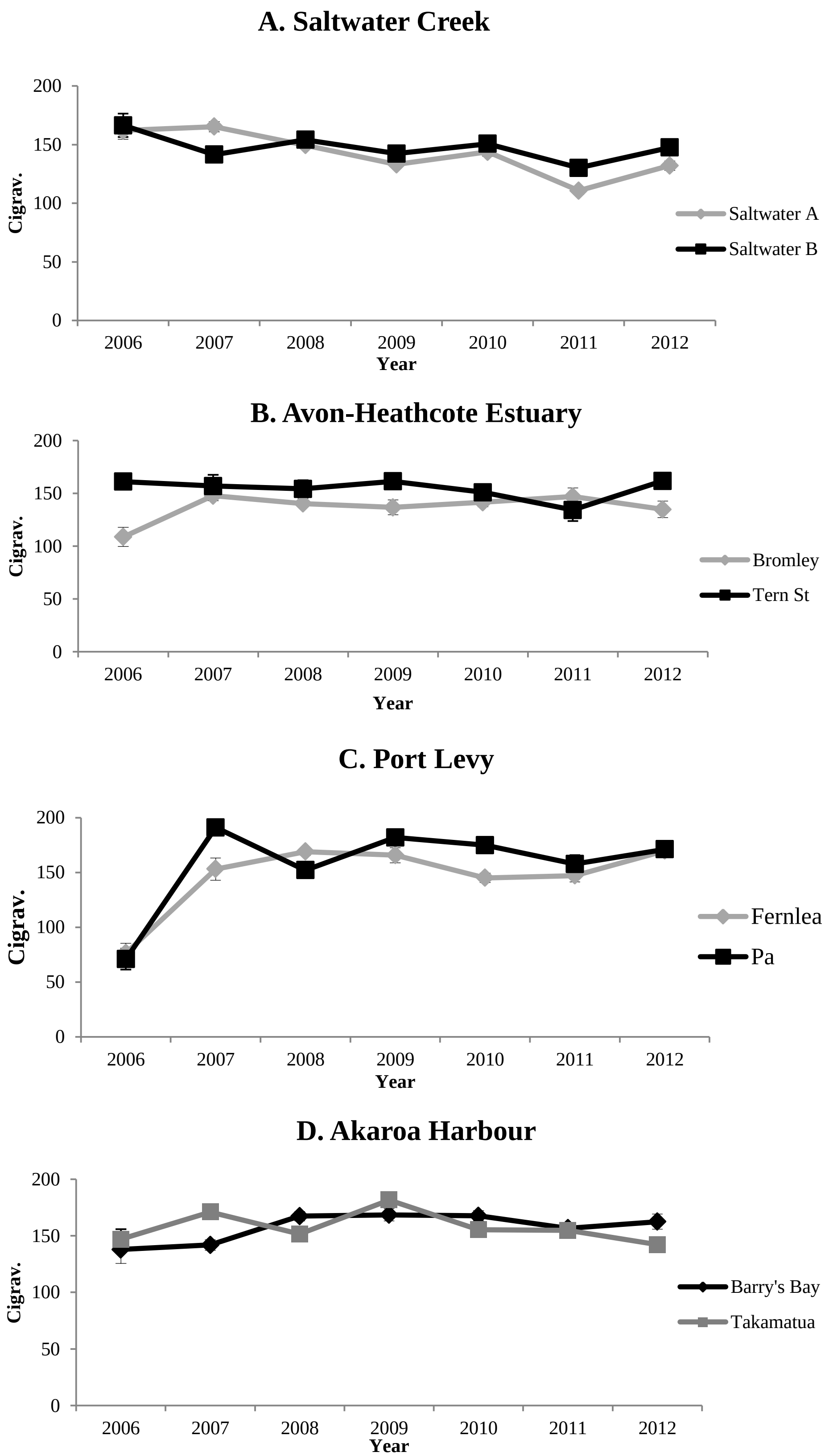


Figure 4.12: Mean condition index (gravimetric) for *Austovenus stutchburyi* from 8 sites over 7 summers. Dark lines represent high salinity sites.

Table 4.20: Results of repeated measures ANOVA comparing seasonal gravimetric Condition index of *Austrovenus stutchburyi* at the 8 sites. Significant values are shown in bold.

Source	***df***	***F value***	***P-value***
Site	7	18.5	**0.001**
Season	12	185.5	**0.001**
Site x Season	84	7.3	**0.001**

Table 4.21: Results of repeated measures ANOVAs comparing gravimetric condition index of *Austrovenus stutchburyi* at high and low salinity sites at each of the 4 estuaries over 13 seasons (Dec. 2006-Jan 2009). Significant values are shown in bold.

Area	**Source**	***df***	***F value***	***P-value***
Saltwater Creek	Site	1	7.24	**0.015**
	Season	12	54.8	**<0.001**
	Site x Season	12	3.3	**<0.001**
Avon-Heathcote	Site	1	16.97	**0.001**
	Season	12	26.99	**<0.001**
	Site x Season	12	1.71	0.067
Port Levy	Site	1	2.47	0.134
	Season	12	47.38	**<0.001**
	Site x Season	12	2.34	**0.008**
Akaroa Harbour	Site	1	0.7	0.407
	Season	12	100.70	**<0.001**
	Site x Season	12	3.50	**<0.001**

Table 4.22 Results of repeated measures ANOVA comparing gravimetric condition index of *Austrovenus stutchburyi* at all sites over 7 summers (2006-2012). Significant values are shown in bold.

Source	***df***	***F value***	***P-value***
Site	7	5.80	**<0.001**
Year	6	20.71	**<0.001**
Site x Year	42	9.65	**<0.001**

Table 4.23: Results of repeated measures ANOVA comparing gravimetric condition index of *Austrovenus stutchburyi* at high and low salinity sites at each of the 4 estuaries over 7 summers (2006-2012). Significant values are shown in bold.

Area	Source	*df*	*F value*	*P-value*
Saltwater Creek	Site	1	6.62	**0.019**
	Year	6	14.63	**<0.001**
	Site x Year	6	3.68	**0.002**
Avon-Heathcote	Site	1	14.76	**0.001**
	Year	6	1.16	0.331
	Site x Year	6	1.69	0.130
Port Levy	Site	1	5.41	**0.032**
	Year	6	53.28	**<0.001**
	Site x Year	6	4.43	**<0.001**
Akaroa Harbour	Site	1	3.38	0.568
	Year	6	5.09	**<0.001**
	Site x Year	6	7.72	**<0.001**

Table 4.24: Spearman rank correlation analysis investigating the relationship of site, season, cockle length and live weight with gravimetric CI.

	Season	*Site*	*Length*	*Whole live wt*	*Cigrav*
Season	1				
Site	0	1			
Length	0.019	-0.043	1		
Whole live wt	**0.128**	**-0.159**	**0.061**	1	
CI grav	**0.149**	**0.852**	**-0.064**	**-0.414**	1

There were significant positive correlations between season and whole live weight and CI gravimetric, and between site and CI gravimetric (Table 4.24). There was also a positive correlation between length and whole live weight. Site and whole live weight, length and CI gravimetric, and whole live weight and CI gravimetric all showed significant negative correlations.

Assessment of the relationship between condition index, glycogen level, gonad index and gonad index (Table 4.25) gave only a significant correlation between gonad index and glygogen levels (Figure 4.9) .

Table 4.25: Spearman rank correlation decribing relationship between glycogen levels, gonad index, condition index and growth rings in *Austrovenus stutchburyi.* Significant values are shown in bold (p<0.05) (N=32).

	Glycogen	Gonad index	CI grav	Growth
Glycogen	1			
Gonad index	**0.458**	1		
CI grav	-0.209	-0.298	1	
Growth	-0.253	-0.346	0.0206	1

Correlations

Table 4.26: Results of Spearman Rank Correlation analysis investigating the relationships between cockle size, density, condition index, trace metals and environmental variables. Correlations in bold are significant (*p*=0.5)

	Site	*Mean size*	*Largest size*	*Density*	*%silt*	*%sand*	*Salinity*	*CIgrav mean*	*Temp*	*TVS*	*Gonad index*	*MPI mean*	*F/M/ratio*	*Human impacts*	*Agri impacts*
Site	1														
Mean size	-0.275	1													
Largest size	-0.167	**0.923**	1												
Density	0.239	-0.591	-0.429	1											
%silt	-0.159	-0.307	-0.494	-0.302	1										
%sand	0.159	0.307	0.494	0.302	**-1**	1									
Salinity	0.590	0.125	0.342	0.024	-0.465	0.465	1								
CIigrav mean	**0.908**	-0.375	-0.164	0.499	-0.279	0.279	**0.704**	1							
Temp	0.007	**0.896**	**0.884**	-0.332	-0.506	0.506	0.189	-0.075	1						
TVS	-0.622	-0.224	-0.161	-0.106	0.159	-0.159	-0.552	-0.585	-0.349	1					
Gonad index	0.164	-0.373	-0.375	0.190	-0.165	0.165	0.129	0.090	-0.482	0.058	1				
MPI mean	0.252	0.346	0.092	-0.513	0.369	-0.369	-0.289	-0.117	0.392	-0.323	-0.266	1			
F/M/ratio	-0.282	-0.092	-0.026	-0.268	-0.078	0.078	-0.340	-0.353	-0.057	**0.808**	-0.076	-0.058	1		
Human impacts	0.357	0.486	0.568	0.220	**-0.748**	**0.748**	0.641	0.453	0.652	**-0.735**	-0.055	-0.070	-0.521	1	
Agri impacts	-0.405	0.0452	0.007	-0.417	0.528	-0.528	-0.505	-0.559	-0.186	0.636	0.082	0.198	0.349	**-0.714**	1

The condition index was positively correlated with site(0.908) and salinity (0.704)(p=0.5) (Table 4.26). Maximum shell length (0.884) and mean shell length (0.896) were positively correlated with water temperature. MPI was not correlated with any biological aspect, but total volatile solids (TVS) were positively correlated with female:male ratio (0.808) and human impacts (0.735).

Discussion

Organisms have to maintain a balance in energy utilization between growth and maintenance and reproduction, with environmental stresses having a definite impact on energy demands (Carmichael et al. 2004; Kilada et al. 2008), with stressed organisms being deprived of the necessary energy for both types of processes. Stress response can occur firstly at the cellular and individual level culminating in population and community impacts (Stewart 2005). By measuring changes at the cellular and individual level such as growth and reproduction, it is possible to identify critical changes to populations including bivalves such as *Austrovenus stutchburyi* and the communities of which they are part. The relationship of the reproductive cycle to glycogen content and condition has been identified as being closely linked (Mayer et al. 1992; Norkko 2005; Stewart 2005) and as such should be a feasible indicator of cockle population viability. Stewart (2005) suggests that a decline in recruitment may be the result of insufficient energy stores to initiate gametogenesis but this is debatable.

The reproductive cycles of male and female cockles at these Canterbury sites showed considerable variation with males being active all year round while female activity occurred mainly in the summer, and so was probably associated with warmer water temperatures. All the sites showed a peak in late active and ripe stages over the summer suggesting that *Austrovenus stutchburyi* in the Canterbury region shows peak breeding activity at this time of year although precise spawning episodes were not identified. Although most breeding activity occurred in the summer, it was not limited to these warmer months with late active or ripe males and females occurring all year to a lesser degree. Stewart (2005) found that there may have been two spawning events at the temperate latitude North Island cockle beds she used in her study. She also states that the timing of the gametogenic cycle is likely to vary from year to year due to environmental influences which is not supported by the findings of this study. Larcombe (1971), studying a larger latitudinal range of cockle beds in the North Island, found gonad development in *Austrovenus stutchburyi* to be similar across all sites, with spawning beginning in January. Marsden (1990) found peak breeding occurred in the summer for the surf clam *Paphies donacina* in Pegasus Bay, a pattern similar to those found for *P.subtriangulata* (Grant & Creese 1995) and *P. australis* (Hooker & Creese 1995). However, it has also been established that in the warmer northern regions of New Zealand gametogenesis is continuous throughout the year with more than one spawning episode (Grant & Creese 1995; Hooker & Creese 1995). Norkko et al. (2006) also found *Austrovenus stutchburyi* spawned over summer, while the surf clam *Paphies donacina* spawned in spring. From this evidence it appears that *A. stutchburyi* performs much like bivalves including clams from other parts of the world, with spawning often occurring over the warmer months, but not limited to these or to a single annual episode.

In the northern hemisphere, venus clams including *Mercenaria mercenaria* (Clapp et al. 2008; Marroquin-Mora et al. 2008), *Fulvia mutica* (Liu et al. 2008), *Cyclina sinesis* (Yan et al. 2010), *Cerastoderm edule* (Boyden 1971) and *Ruditapes decussatus* (Anibal et al. 2011) have all been found to spawn during the summer when water temperatures are warmer, although Heffernan et al. (1989) identified two to three spawning peaks in *M. mercenaria* in

Wassaw Sound, Georgia, along with variable reproductivity over consecutive years. Hesselman et al. (1989) report a bimodal spawning pattern (spring and autumn) for *Mercenaria* spp in Florida with large numbers of clams in a resting or spent phase when summer water temperatures could be higher than 30°C. Cobb et al. (2011) also report *Donax variabilis* (Coquina clam) in the Gulf of Mexico as having a continuous reproductive cycle with spawning peaking in spring, although other researchers (Jones et al. 2004) have found variations related to population location. Rodriguez-Jaramillo et al. (2008) also identified a temperature relationship between maturation and spawning in the oyster *Crassostrea corteziensis* in N.W. Mexico, although this species appears to have two spawning episodes, in summer and autumn.

The present study (April 2006-January 2007) found that summer gonad indices were the same for all sites except Fernlea (Port Levy/Koukourārata). This site had a consistently lower gonad index than its higher salinity partner site (Pa). At the low salinity site in Akaroa Harbour (Takamatua) the gonad index was consistently higher than at Barry's Bay supporting previous evidence (Stewart 2005) that cockles from impacted sites reach reproductive maturity at smaller sizes probably through diverting energy from growth to gamete production. Other species have also demonstrated this strategy (Stewart 2005). Larcombe (1971) suggests that only cockles greater than 18mm in length are capable of breeding, regardless of age, but this study does not support this with individuals smaller than 18mm (i.e.15mm) being staged as reproductive. Oocyte numbers in female cockles varied significantly between the 4 areas, and within these areas between the paired sites except for Port Levy/Koukourārata. There were seasonal differences in the oocyte diameter between the sites except for Port Levy/Koukourārata, with the largest being seen mainly in the summer, and smaller ones over autumn and winter. A similar size pattern in male follicles was also observed with seasonal variation in numbers of follicles. The ratio of male to female cockles was not always even, based on a small data set (n=10 for each site). This result differs to that recorded by Stewart (2005).

Through determining age and growth of *Austrovenus stutchburyi* by counting annual growth rings on sectioned cockle shells, there were significant differences between all the sites. Growth patterns were variable in some sites where no correlation was found between shell length and the number of rings indicating that growth is site specific. Comparison of shell growth at high and low salinity sites also revealed differences at three of the four areas, Saltwater Creek, Port Levy/Koukourārata and Akaroa Harbour. Salinity levels have been identified as having an influence on metabolism and growth in bivalves (Marsden 2004; Wang et al 2011) but this study does not conclusively support this as although growth was low at two of the low salinity sites (Saltwater Creek A and Takamatua) it was not at the other two (Bromley and Fernlea). Low growth was also recorded at one high salinity site (Barry's Bay in Akaroa Harbour). This result suggests that salinity levels are not the sole factor controlling population dynamics and that other factors such nutrient availability, temperature and sediment structure should also be taken in account. That shell length showed a significant positive correlation with water temperature can be explained by warmer conditions enhancing nutrient availability thus optimising nutrient intake.

In this study, glycogen levels in cockle foot muscle varied seasonally between the eight sites, but within the four areas, cockles at the paired low and high salinity sites did not show significant differences except for the sites in Akaroa harbour where the higher salinity Barry's Bay site had significantly higher levels of foot muscle glycogen than those at Takamatua. All the sites except Saltwater B and Fernlea showed an increase in glycogen levels over the summer, with these two sites peaking later in autumn. The lower glycogen levels leading up to summer support summer spawning with energy being channelled into gamete production in the months before. Glycogen levels did not clearly distinguish between sites, and as such would not be a fool-proof method for identifying sites with differing environmental qualities (De Luca-Abbot 2000, Stewart 2005). In fact, the anomaly apparent when comparing the glycogen levels for the 8 sites is Barry's Bay, which had higher values than any of the others. This is a higher salinity site which recorded low cockle growth and where the sediment is classed as silty (Chapter 3). This re-enforces the concept that no one environmental variable should be used to determine the controlling population dynamics.

Condition index (gravimetric) showed significant spatial and seasonal differences. Salinity is recorded as being an influence on CI along with other environmental influences such as temperature, nutrient availability as well as by population dynamics (Marsden & Pilkington 1995; Marsden 2000; Norkko 2005; Stewart 2005). The fact that significant differences in CI (gravimetric) are apparent between the high and low salinity paired sites supports the role of salinity as an environmental stressor, a conclusion that may not have been identified if CI was assessed using the CI dry formula. All the sites show a marked CI peak at the autumn 2006 sampling which cannot be explained by the research data. It may have been that during the preceding summer nutrients were readily available, or it may be that spawning was delayed. In both these scenarios energy may have been conserved, leading to the increased condition indices across all sites.

This study has not revealed a clear relationship between environmental factors and the growth and reproduction of *Austrovenus stutchburyi* in the Canterbury region of New Zealand. While it has identified a correlation between salinity and condition index and oocyte diameter, it has not established a clear cut relationship between salinity and growth and glycogen levels. Taking the view that reduced growth is linked to poor environmental conditions (or contamination) one could question why the Takamatua site where the population was dominated by small cockles had the highest gonad index while glycogen levels and condition indices were not hugely different from the other seven sites. There was no one environmental factor standing out as having an effect on this site. Perhaps it is a case of channelling energies into reproducing at the expense of using energy to grow. This is an avenue for further research.

The findings of this research, which suggest that the optimum time for spawning is as the weather is warming, can be linked with the environmental characteristics of these sites to predict that the optimal time for transplanting cockles falls in the same time frame. With water temperature increasing in spring and increased levels of micro-organics, there is an increased nutrient source to support an influx of new individuals, whether they are new recruits or transplants.

Chapter 5

Cockle transplantation and restoration

5.1 Introduction

Degradation of natural coastal habitats is occurring worldwide as a result of anthropogenic impacts such as altered land use, with resulting sedimentation, nutrient change and contamination leading to decreased shellfish stocks (Fegley et al.1992; Navarro et al. 1992; Stewart & Creese 2002; Tallis et al. 2004; Cummings et al.2007; Marsden & Adkins 2010; Ravit et al. 2012). These decreases in turn impact on the general ecosystem functioning. As a result of this, research carried out worldwide to establish criteria for successful restoration or enhancement of degraded habitats (Negrello Filho et al. 2006; Johnson & Heck 2007; Doall et al. 2008; Lundquist et al. 2009), has identified that processes are species and site specific (Cummings et al. 2007) with the timing of the transplant operations and the size of the bivalves being very important. Specialised knowledge is required of the ecology of the species involved and the factors such as water and sediment quality, and nutrient availability which impact on survival and growth. Overharvesting also impacts on shellfish population dynamics, resulting in smaller individuals and lower densities (Stewart & Creese, 2002: Stewart 2005).

In New Zealand, environmental stressors all impact on cockle health, and consequently the reproductive and recruitment potential. Sedimentation alters extraction of phytoplankton from the water and so may change nutrient availability (Iglesias et al, 1992; Navarro et al, 1992), and can be associated with the introduction of contaminants. These factors may also be linked to eutrophication which may have deleterious effects, although Shriver et al (2002) determined that the land–derived nitrogen load did not alter the condition of bay scallops and the additional nutrients may have resulted in higher growth rates. Sedimentation can also affect water current regimes which can lead to a reduction in seston concentrations in the near bottom layers (Marsden, 2004; Dolmer, 1999).

Increasing water temperatures can impact on reproductive output because energy is diverted from gonad development to general metabolic processes to cope with the warmer environment. In addition, the reduced oxygen-carrying capacity of warmer water in conjunction with sedimentation (Ellis et al 2002) will result in reduced gas exchange efficiency which in turn will impact on the general metabolism of the animal. Predation impacts on larval recruits (Beukema and Dekker 2005) which can be related to a warming climate, as well as to sediment changes can lead to declining densities. Cockle mortality has been shown to increase through parasitism with infestation causing the bivalves to come to the substrate surface while feeding (Desclaux et al, 2002) making them more accessible to predators. Parasite infection may also contribute to increased uptake of contaminants. Research with northern hemisphere species (*Cerastoderma edule (*cockle), *Ruditapes philipparum* (clam) and *Crassostrea gigas (*oyster)) showed that cockles infected with a digenean trematode had increased concentrations of contaminants (cadmium, zinc and copper) in their tissues (Baudrimont et al, 2005). It has been documented that the New Zealand cockle *Austrovenus stutchburyi* is prone to trematode infestation of the foot (Mouritsen 2002) which reduces the burrowing ability of the animals, leaving them vulnerable to predation. As well, a relationship has been established between parasite infestation and cockle condition (Mouritsen & Poulin 2003), with poor condition being linked with greater parasite numbers.

One approach to rectifying shellfish stock reductions is by implementing harvesting bans. In New Zealand bed closures are achieved through several levels of marine protected areas with the highest protection being attained through marine reserves. Being a small country with an extensive coastline New Zealand has less than 1% of the coastline protected even though there are currently 18 fully protected marine reserves on the mainland. Ngāi Tahu manage the shellfisheries in the South Island of New Zealand using three levels of closures - rāhui, mātaitai and taiapure. A rāhui restriction, imposed by the Minister of Primary Industries by way of the Fisheries Act 1996, limits fishing in an area for up to two years in order to restore the customary mahinga kai food resource. A taiapure is an area identified by iwi (local tribe) or hapū (sub-tribe) as a source of food or for spiritual or cultural reasons and is managed by a community management committee including representatives from environmental groups, commercial fishers, tourism and marine farmers (Pirker 2008). A mātaitai designates an area as important for customary fishing and allows tangata whenua (descendants of the first people to settle the land) to manage the area permanently. Examples of this type of reserve on Banks Peninsula occur at Rapaki and at Koukourārata (Port Levy), a study site for this thesis.

Pre- and post-settlement processes are important structuring agents of invertebrate populations and communities (Menge, 2000; Todd 1998; Olafsson et al.1994). Predation is documented as having large impacts on dispersing larvae (Hunt & Scheibling, 1997; Andre & Rosenberg, 1991) and along with competition and adult juvenile interactions (Thrush et al, 1996), physical disturbance, sediment pH (Green et al, 2004, 2009) and physiological stress will lead to increased mortality on newly-settled larvae (Todd, 1998). Also, dispersal pathways and pelagic larval durations (PDL) play a role in the successful recruitment in bivalve beds with genetic division occurring between regions if there are barriers such as water current up-or down-wellings and short PDLs (Ross et al. 2009). Using mitochondrial CO1 Ross (2011) identified six divergent subpopulations for *Austrovenus stutchburyi* through central New Zealand, about North and east Capes and in southern region of the South Island which has a PDL of 2-5 weeks.

While natural recruitment may lead to the recovery of cockle beds, it appears that harvesting bans alone may not be effective in the short to medium term (Stewart & Creese 2002) and that bed enhancement may be necessary. Enhancement of an environment can be achieved by modifying the existing habitat to make better use of natural and artificial larval recruitment, and also by introducing mature individuals to boost natural larval recruitment (Morrison & Browne 1999). It has been established recruitment for *Austrovenus stutchburyi* is extremely variable (Larcombe 1971).

Habitat restoration is not often a realistic option because many of the changes are of such a scale that they cannot be reversed. However, by re-establishing depleted species after the introduction of environmental regulations to limit the negative anthropogenic impacts, an ecosystem can be improved both from economic (e.g. shell-fishery) and ecological perspectives. Benthic bivalves such as clams, cockles and oysters not only contribute to the physical stability of an area, they filter large quantities of water, which leads to improvements in the water quality (Ravit et al. 2012).

Research to date on *Austrovenus stutchburyi* shows that transplantation is possible using adult cockles with lesser success being achieved using juveniles. Preliminary research in Whangateau Harbour (Stewart 2002) using manipulative field experiments looked at factors affecting growth and survival of transplanted individuals, and also investigated the seasonal timing for successful transplantation. Further research in this region continued on the effects of urbanisation on cockle populations (Stewart 2005). Factors that impact in this region close to Auckland city may be quite different to those that effect shellfish populations on South Island east coast beaches. In North America successful enhancement of estuarine bivalve populations have been achieved through the out-planting of hatchery-produce spat and in Spain, Holland and United Kingdom cockle reseeding using juveniles is practiced in commercially fished beds (Gosling 2003).

The aim of this research was to investigate various transplant options with a view to developing protocols for the efficient and economical transfer of adult cockles from healthy beds to those showing population reductions. It was hypothesised that large cockles (25-35mm length) confined in 300mm square cages at medium densities (50 cockles/cage) would have the greatest potential for survival over a 12 month period because they would cope with the stress of handling, and being enclosed in cages, would be protected from predators. This degree of protection would also allow assessment of the necessity of implementing habitat closures to optimise the success of transplanting exercises. Because an understanding of population connectivity is important for responsible management and enhancement of ecosystems, especially when employing transplantation procedures, a small sub-sample of South Island cockles was analysed for mitochondrial cytochrome *c* 1 haplotypes with the premise that there may be limits on the availability of sources for cockles for transplanting based on sub-population differences.

5.2: Methods

In the first trials, January 2006 through to January 2007, plastic 170mm square, 2 litre containers with 10mm holes punched in the bottom, sides and lid were trialled. These were modified with mesh panels in the bottom and lid to facilitate water flow. However, neither option allowed long term survival of the cockles. There was considerable scouring of the sediment around these cages, and the cockles died within 3 months of transplanting, perhaps due to extremes of temperature, lack of oxygen, and lack of nutrients.

Preliminary Transplant Trial between estuaries

At the end of November 2007, as a preliminary trial, cockles of three length groups (small, <10mm, medium, 15-20mm and large, 25-30mm) sourced from Beachville Road in the Avon-Heathcote/Ihutai Estuary were placed in duplicate perforated plastic containers at three sites, Saltwater Creek, Tern Street and Takamatua with a total of 30 cockles per cage. Duplicate controls using locally sourced cockles were set up at each site using the same class sizes as for the transplants. Sediment from each of the cage sites was sieved using a 2mm mesh sieve, all animals were removed and the cleared substrate used to back-fill the cages. These were left for 12 weeks, then removed, and the substrate sieved to separate the test and control cockles. Surviving cockles were returned to the laboratory for analysis of condition index along with un-caged natural cockles from all trial sites as well as un-caged cockles from the Beachville Road donor site.

After preliminary trials using various methods of caging cockles, ‘Gutter guard’© was selected as the most effective material for making cages, being easy to manipulate and inexpensive.

Short Term Closed Cage Transplant in Avon-Heathcote/Ihutai Estuary

A trial using 200mm square by 100mm deep cages constructed entirely of gutter guard mesh (Figure 5.1) commenced in mid-December 2008. Cockles of two class sizes (15-20mm, and 25- 30mm) were collected from the mid-tide area in the Avon-Heathcote/Ihutai Estuary adjacent to Plover St. These were transported to the lab in chilly bins and held overnight in the aquarium, before being returned to the two study sites in the mid-tide region of the estuary, a high salinity one (35ppt) at Plover St (S 43° 32.927´, E 172° 44.559´) and the other at the low salinity (28ppt) Bromley outlet (S 43° 32.488´, E 172° 43.279´). The sites were set up with 3 rows of 4 cages with a central post at each site carrying a temperature data logger. The substrate was excavated to a depth of 50mm and a cage placed in position with the cage top flush with the top of the sediment. Fifteen cockles of each size class were placed in each cage, a total of 30 cockles per cage. Clean sieved substrate from each site was used to fill the cages.

Figure 5.1: Cage constructed from plastic mesh

In mid-February 2009, the first of the 4 planned monitoring exercises took place. Three randomly selected cages from each site were lifted and the numbers of live, dead and missing cockles recorded. 5 cockles were taken from each of the 3 cages at each site for condition analysis. Naturally occurring cockles were gathered as condition controls at both sites. At the Plover St site, 8 cages were visible with the other 4 completely buried in the substrate. At Bromley, parts of all the cages were visible.

The second monitoring exercise was carried out at the end of March 2009 when a further 3 randomly selected cages were lifted at each site and the live cockles taken to the lab for condition analysis along with natural control animals. The three cages lifted at both sites at the 3rd monitoring session in May 2009 contained no live cockles, so the final 3 cages were lifted, again with no live cockles found. There was no 4th monitoring session.

Long Term Open Cage Transplant at Plover Street

A further transplant trial was established in the Avon-Heathcote/Ihutai Estuary in October 2009. Two sites, approximately 250m apart, were set out in the mid tide area adjacent to Plover St with the expectation that even placed as close as this, there would be differences in the condition and survival of the transplanted cockles between the two sites by the end of the experiment. The sites measured 31.2m by 16.2m with 4 rows of 4 plots in each. Rows were separated by 5m each and plots within rows by 10m (Figure 5.2). Temperature loggers were place on wooden stakes at the centre of each site, and these also provided points for GPS referencing. The eastern site (Site 1) was located at S 43° 33.029´, E 172° 44.496´ and the western one (Site 2) at S 43° 33.052´, E 172° 44.362´. At each site, sixteen 300mm x 300mm plots were marked out prior to excavation to a depth of 50mm. The removed sediment was sieved through a 2mm mesh sieve and all *Austrovenus* and other species were counted and recorded. Eight 'gutter guard'© cages were placed in position along with 8 uncaged plots. These cages were mesh 'fences' with no bottom or top covering to allow the cockles to move vertically within the sediment.

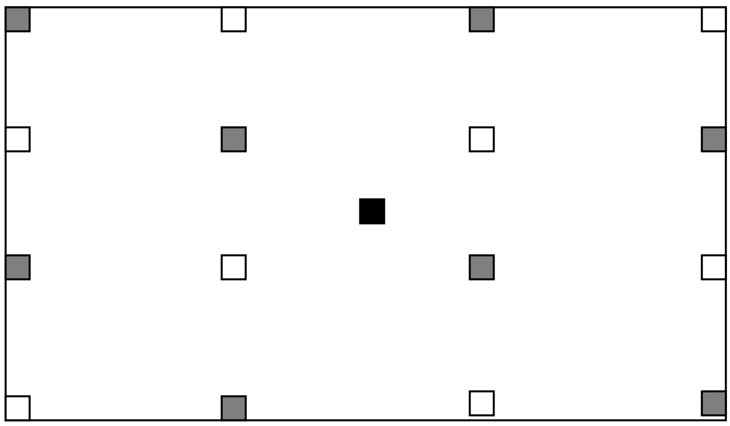


Figure 5.2: Experimental design for open caged transplant experiment.

Key: **Caged plot** **Un-caged plot** **Data logger**

Cockles for transplanting were sourced from Beachville Road cockle bed at the southern edge of the Avon-Heathcote Estuary (size range 25-30mm shell length) the day before the transfer exercise. These were held in a cool room to allow the shell to dry prior to spray painting on the day of transplant operation (Figure 5.3).

Figure 5.3: ***Austrovenus stutchburyi*** **in the process of being spray-painted prior to transplanting.**

At the estuary, 50 painted cockles (Figure 5.4) were placed in each of the excavated plots and covered with the sieved sediment. Eight replicates of caged and un-caged treatments were established at each site.

Figure 5.4: Painted cockles in an open cage prior to covering with sieved sediment

As a control, a back transfer of cockles was set up at the Beachville Road site. In the mid-tide region, it was a different layout to the experimental plots. Beachville Road is very accessible to the public, and the experimental area needed to be as inconspicuous as possible, so no marker stakes or cages were used and the area was reduced, being 3m by 2m. This was divided into six $1m^2$ plots with 40 cockles regularly spaced in each.

Monitoring

Assessment of survival and growth (but not condition) of the transplanted cockles were carried out at 6-9 week intervals beginning in December 2009 and continued until the final lift in November 2010. At each monitoring session, 2 of each of the caged and un-caged replicate plots were randomly selected, and the cockles were collected by hand sifting of the sediment. Counts of both live and dead marked *Austrovenus* were made. Missing animals were recorded and also any unmarked individuals in each plot. Movement of marked cockles from the un-caged plots was assessed by counting the number of marked cockles found in a 0-15cm and 15-30 cm area immediately surrounding each original plot. Dead transplanted cockles were counted and collected.

At the control site, four 150mm x150mm quadrats were randomly sampled within the six $1m^{2\ plots}$. The number and size of any marked cockles found were recorded, and they were returned to the laboratory for condition analysis, along with a corresponding number of naturally occurring cockles collected adjacent to the control plot. 25mm diameter by 20mm deep sediment cores were collected from each plot at all sites to determine grain size, organic content, and pore water. Water samples were collected on the in-coming tide for chlorophyll α content.

Sediment analysis.

The core samples were homogenised and sub-samples used to determine pore water, grain size and organic content. Pore water was determined by drying a pre-weighed sub-sample at 60°C for 72 hours and then reweighing the sample. The percentage of pore water was calculated using the formula (wet weight-dry weight)/ wet weight x 100. Loss of volatile solids was used to determine organic content. Each dried sample from the pore water analysis was ashed at 450°c for 5 hours and then reweighed. The percentage of volatile solids was calculated as (pre-ashed weight-post-ashed weight)/pre-ashed weight x 100. The samples were then broken up using a pestle and mortar and dry sieved using Wentworth sieves to determine grain size.

Final lift.

In late November 2010, all the transplanted cockles were retrieved from both tests sites and the control site. Sediment was collected from each of the 300 x 300mm plots within the test sites to a depth of 50mm. This was sieved through a 2mm mesh and all the cockles counted and measured for length. Two of each of the transplanted and natural population cockles were collected from each plot for condition analysis. As controls, naturally occurring cockles were collected from four plots both within and outside the site boundaries. At the un-caged plots, an area $1m^2$ was forked through to check for transplanted cockles that may have moved out from where they were originally placed. All the remaining transplanted cockles were removed from the test area and all dead transplanted cockles were collected. Other species present in the plots were counted and recorded.

At the Beachville Road site, the boundaries of the metre-square plots were re-established and two randomly selected 300mm x 300mm quadrates in each of these six bigger areas were excavated, the sediment sieved and painted cockles counted. Two transplanted cockles from each plot were returned to the lab for condition analysis (n=12) along with naturally occurring ones found within and adjacent to the trial plot (n=16 for natural controls).

Mitochondrial Cytochrome C Oxidase

A small subsample of cockles (13 in total) collected from Otago harbour, Rapaki (Lyttleton harbour) and the Pa and Fernlea sites at Port Levy were compared with an Akaroa specimen by Dr. Phillip Ross (Waikato University) following the procedures for the mitochondrial cytochrome c oxidase subunit 1(CO1) gene described in his thesis (Ross 2011) to determine whether the points of origin for these cockles were genetically subdivided, with the results being presented as a cladogram.

Data analysis

Data were analysed for site-specific and between site differences and for treatment differences using general linear models (ANOVA) using STATISTICA© 6. Data were not transformed. The relationship between survival time, condition indices and environmental variables (salinity, nutrients, pore water, sediment and temperature) were tested using Spearman Rank correlation analysis (p = 0.5).

Results

Preliminary Trial

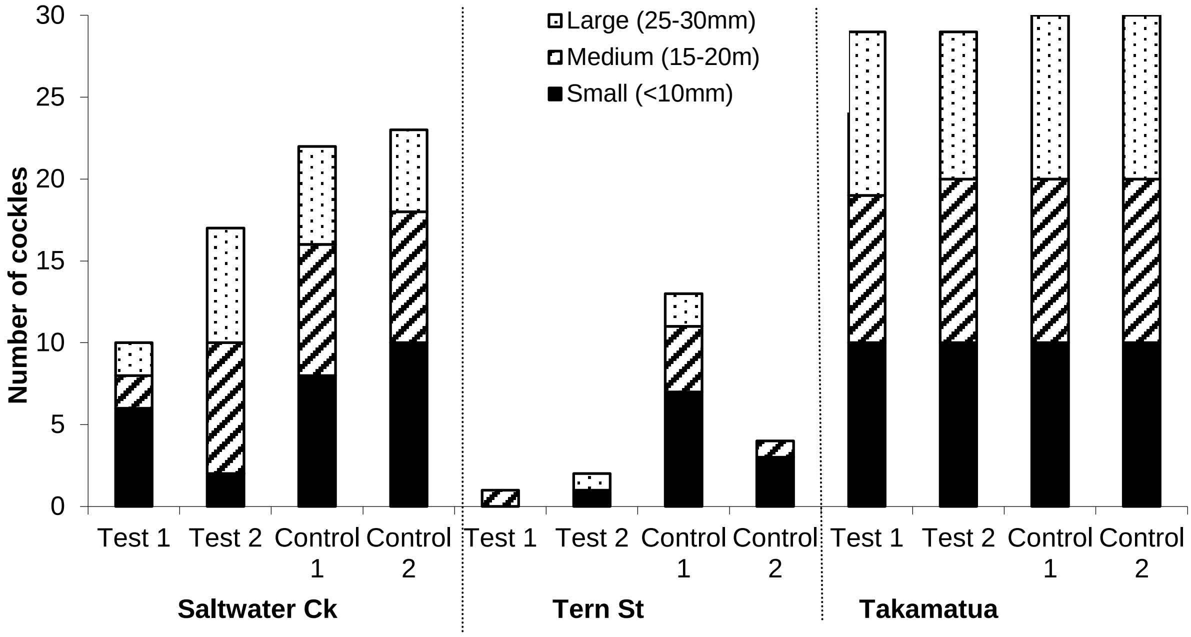


Figure 5.5. Survival results of 3 transplant experiments with cockles sourced from Beachville Road (Avon-Heathcote Estuary) and moved to Saltwater Creek, Tern St and Takamatua and site specific control cockles, November 2007- March 2008. N=10 initially added for each class size for each treatment.

In this preliminary 12 week trial there were significant differences in the survival rate of the cockles across the three sites (p <0.001) with Tern Street (a high salinity site) having the lowest survival rate and Takamatua (low salinity) the highest (Figure 5.5). At saltwater Creek and Tern Street, cockles sourced from Beachville Road (the test cockles) survived less well than those naturally occurring on-site at the 3 trial areas.

There were significant differences (p < 0.001) in the gravimetric condition indices (Figure 5.6; Table 5.1) between transplanted and control cockles, with all transplanted cockles being in poorer condition than the controls from Beachville Road (CI =180). Uncaged site-specific controls were in better condition than their caged counterparts at all sites. The condition of the transplanted cockles at the three test sites was not markedly different.

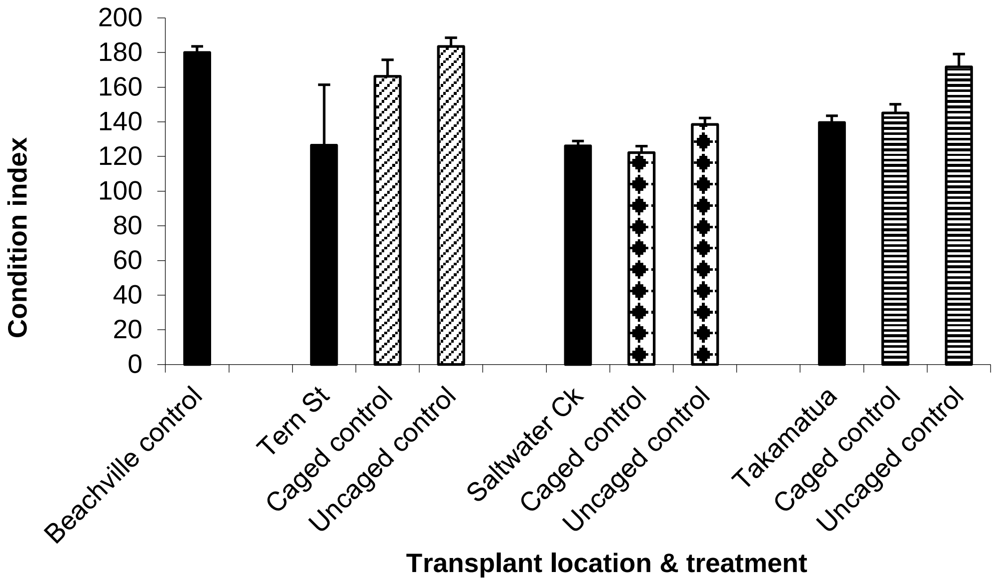


Figure 5.6: Mean condition index (±1SE) for surviving transplanted and control cockles at 3 sites November 2007-March 2008 at the end of the experiment. Uncaged and Beachville controls were collected at time of transplant retrievals. Key to cockle source:
Beachville Road Tern Street Saltwater Creek Takamatua

Table 5.1: One way ANOVA comparing condition index of transplanted and control *Austrovenus stutchburyi* at three sites November 2007-March 2008. Significant values are shown in bold.

Source of Variation	*df*	*MS*	*F*	*P-value*
Between Groups	5	29527.78	11.53	**0.001**
Within Groups	54	2561.69		
Total	59			

Short Term Closed Cage Transplant in the Avon-Heathcote/Ihutai Estuary

This experiment, planned to run from December 2008 through to July 2009, terminated in May 2009 with the total demise of the caged transplanted cockles.

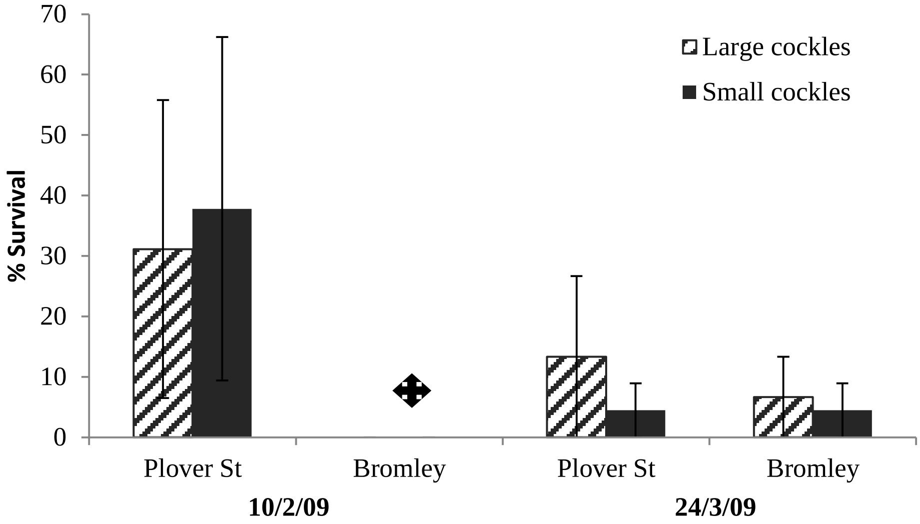


Figure 5.7 Mean cockle survival (±1SE) from the caged transplant trial using cockles sourced fro Plover Street and placed at Plover Street and Bromley, November 2008- March 2009.❖ No live cockles were present in the selected cages at the February 2009 sampling time.

During the time that the experiment was run, there were no significant differences in the survival of either the large or smaller cockles (Figure 5.7; Table 5.2) although the rate of survival decreased between sampling times, with no live cockles being found in the cages in May 2009. By March 2009 the survival rate was better for the larger cockles at Plover Street than at Bromley.

Table 5.2: ANCOVA results describing the survival of transplanted and control cockles from caged transplant trial November 2008-March 2009.

	df	MS	F	*p-value*
Site	1	48.17	3.51	0.077
Time	1	13.50	0.983	0.334
Cockle size	1	0.17	0.012	0.913
Time*Cockle size	1	2.67	0.194	0.664

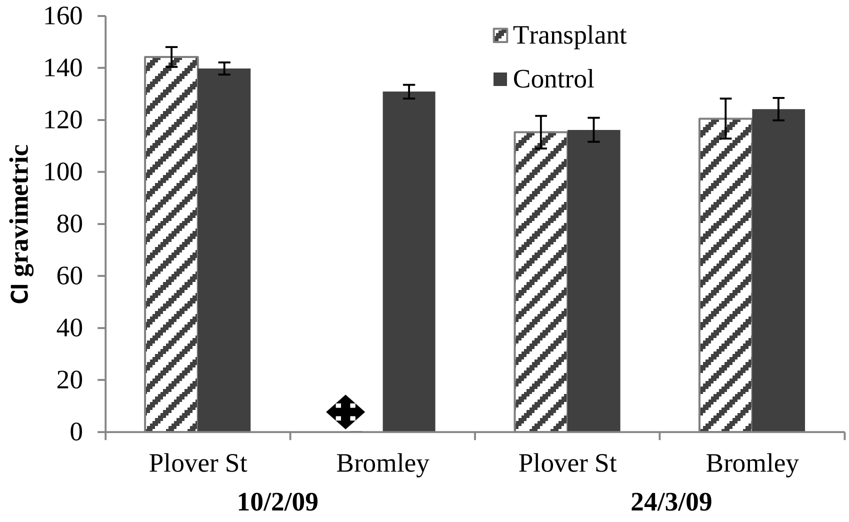


Figure 5.8: Mean condition index (CI gravimetric) (±1SE) at the two productive sampling times for transplanted and control cockles from the caged transplant trial November 2008-March 2009. No live cockles present.

Although there was a significant difference in cockle gravimetric condition index (Table 5.3) over time (p = 0.001) with a decrease from one sampling time to the next, there were no significant differences in condition between the transplanted cockles and the controls (Figure 5.8) at either the Bromley site in March 2009, or at both sampling times at Plover Street.

Table 5.3: Repeated measures ANOVA comparing CI gravimetric in transplanted and control cockles from the caged transplant trial. Significant values are shown in bold.

Source of Variation	*Wilkes value*	*F*	*Effect df*	*Error df*	*P*
Site	.859	2.72	2	33	0.081
Time	.610	10.54	2	33	**0.001**
Time x site	.767	5.02	2	33	**0.013**

Long Term Open Cage Transplant at Plover Street

Invertebrate diversity

Austrovenus stutchburyi was the most common species found in the transplant sites prior to the seeding exercise in October 2009 with approximately 800 cockles /m^2

at site 1 (east) and 350/m^2 at site 2 (west) (Figure 5.9). The pipi, *Paphies australis* was the next most common species at site 2, followed by *Diloma subrostrata*, assorted polychaete worms and assorted crabs. At site 1, these four species were present at similar densities. At the time of the final lift in November 2010, *Austrovenus* was the dominant animal in the excavated plots at all sites, again followed by *P. australis* and *D. subrostrata* (Figure 5.10). Polychaetes and crabs were replaced in 4th and 5th positions by *Cominella glandiformis* (whelk) and the limpet *Cellana radians.* There were significant differences (ANOVA, p = 0.011) in the densities of *Austrovenus* at both sites pre- and post-transplant. There were also significant differences (ANOVA p< 0.000) between the transplant plots and the control plots.

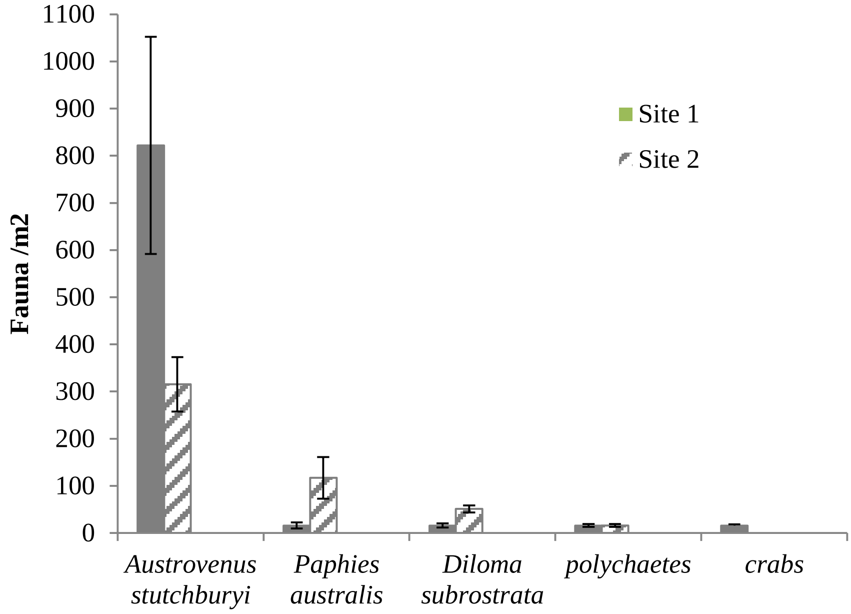


Figure 5.9: Density of the five most common invertebrate species (±1SE) gathered from the transplant plots at the two Plover Street sites prior to seeding of cockles, October 2009 (N=16 for each site).

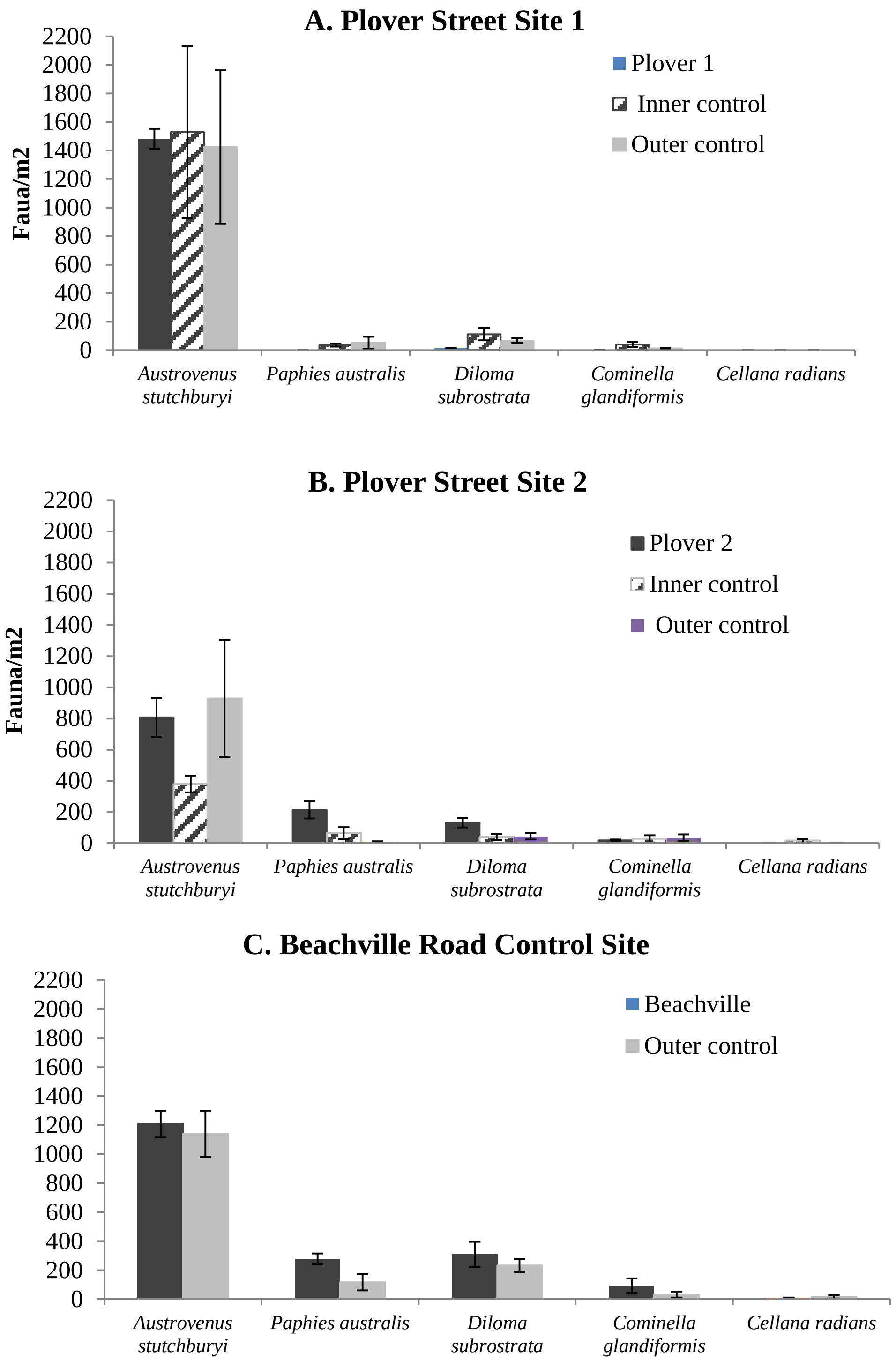


Figure 5.10 A-C: Density (±1SE) of the five most common species at the Plover Street transplant and Beachville road control sites at the time of the final lift, November 2010 (N= 16 for Plover sites 1 and 2; N=4 for site 1 and 2 controls; N= 12 for Beachville Road). N.B. Cockles are naturally occurring ones at each site.

Transplant survival

There were significant differences in the survival rate for both caged and un-caged cockles at each sampling time (Figure 5.11A; Table 5.4) with fewer cockles being retrieved at site 2 (west) at all sampling times for both treatments (approximately half that of site 1 (east)). Overall, caged transplanted cockles had a higher rate of survival than uncaged ones, with site 1 having a better survival rate (average 89%) than site 2 (average 63%) (Figure 5.11). More cockles were missing from the plots (Figure 11B) than were found dead within the plots (Figure11C) with the uncaged plots showing higher losses.

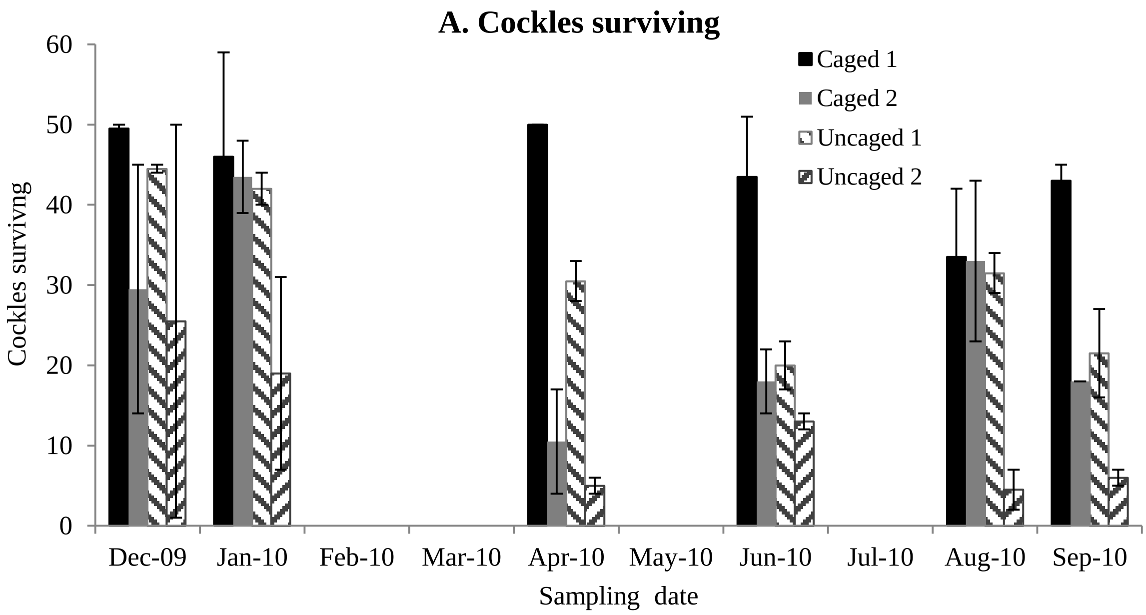
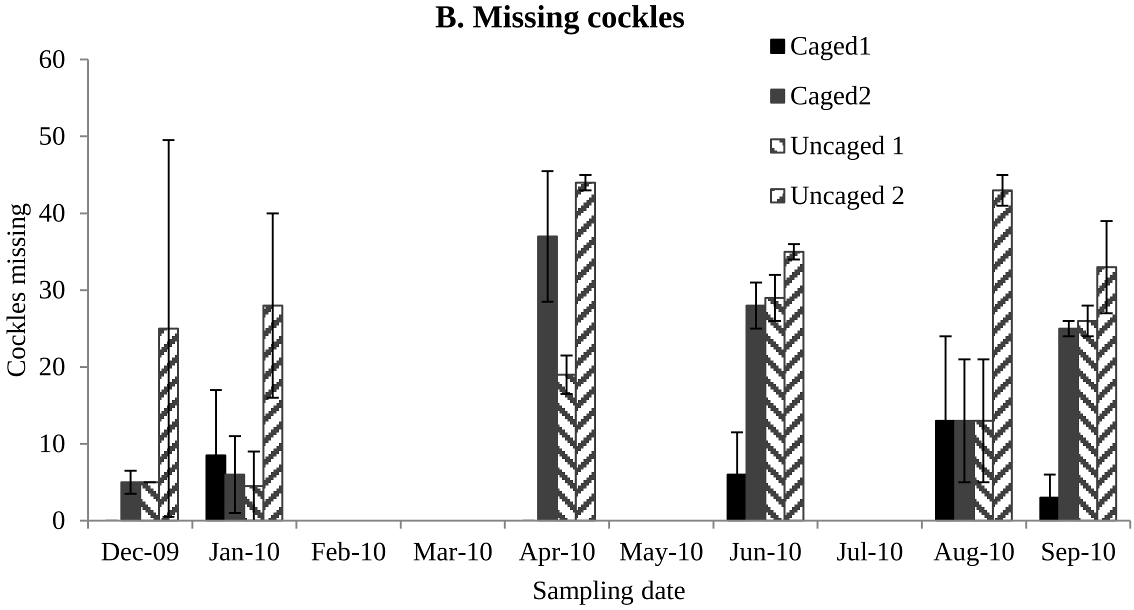


Figure 5.11: Plover Street transplant experient.



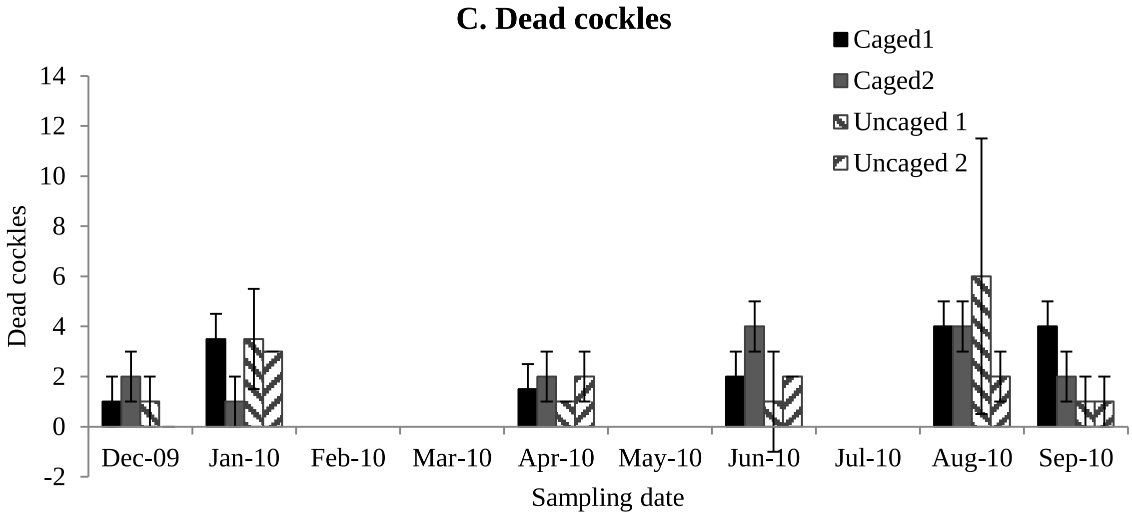


Figure 5.11: Plover Street transplant experient. A, number of live cockles, B, number of missing cockles and C, number of dead cockles (±1SE) in duplicate caged and un-caged plots sampled at 6-9 week intervals at the two transplant sites at Plover Street. Original transplant density for each test plot = 50 cockles.

Table 5.4: ANOVA describing differences in cockle survival between sites and sampling time for the open cage transplant trials. Significant values are in bold.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Time	1010.63	5	202.13	3.84	**0.019**
Site	3205.88	3	1068.63	20.29	**0.001**
Error	790.13	15	52.68		
Total	5006.63	23			

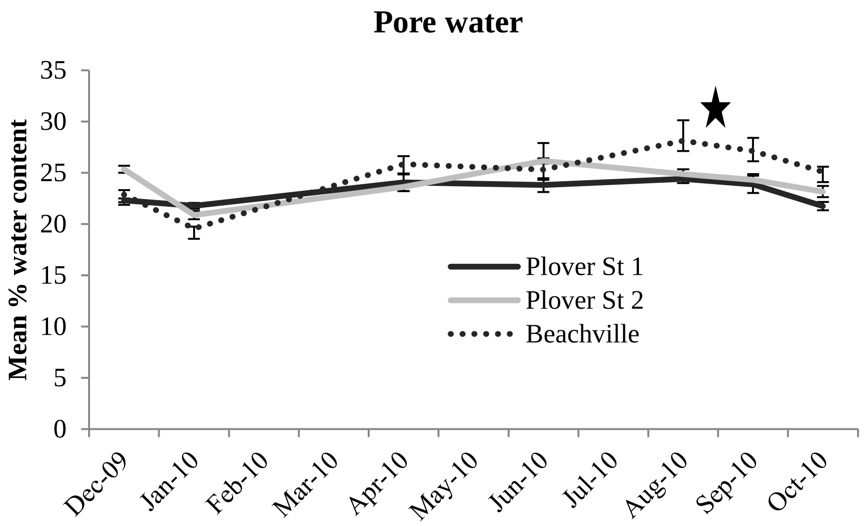


Figure 5.12: Mean percentage pore water content (± 1SE) from the Plover Street transplant and Beachville Road control sites for the trial period, December 2009-October 2010.
★ September 4th 2010 earthquake.

There were significant differences in the mean pore water content both between sites and over time (Table 5.5) with all sites showing a drop after the September 2010 earthquake (Figure 5.12).

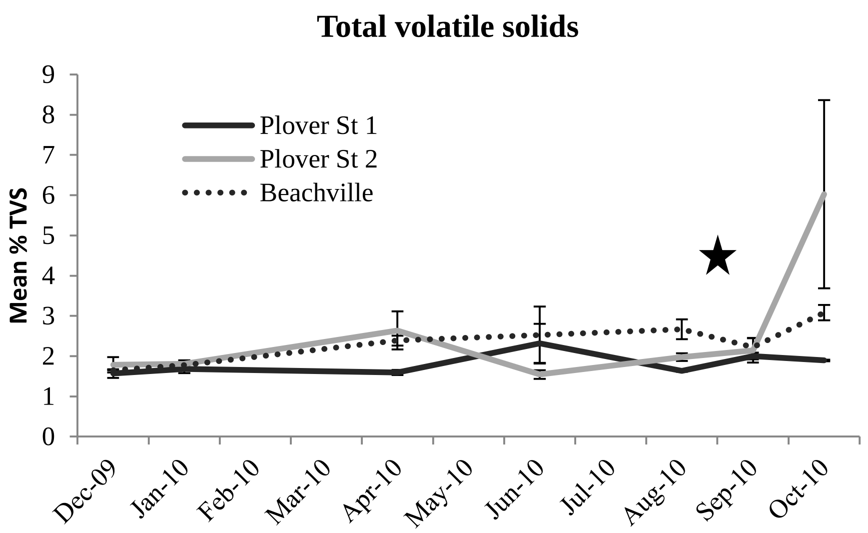


Figure 5.13: Mean percentage total volatile solids (± 1SE) from the Plover Street transplant and Beachville Road control sites for the trial period, December 2009-October 2010.
★ September 4th 2010 earthquake.

There were no significant differences in total volatile solids between sites, but there was one over time (Table 5.5). The control site at Beachville generally had the higher values and both this site and transplant site 2 showed an increase in TVS after the September 2012 earthquake (Figure 5.13).

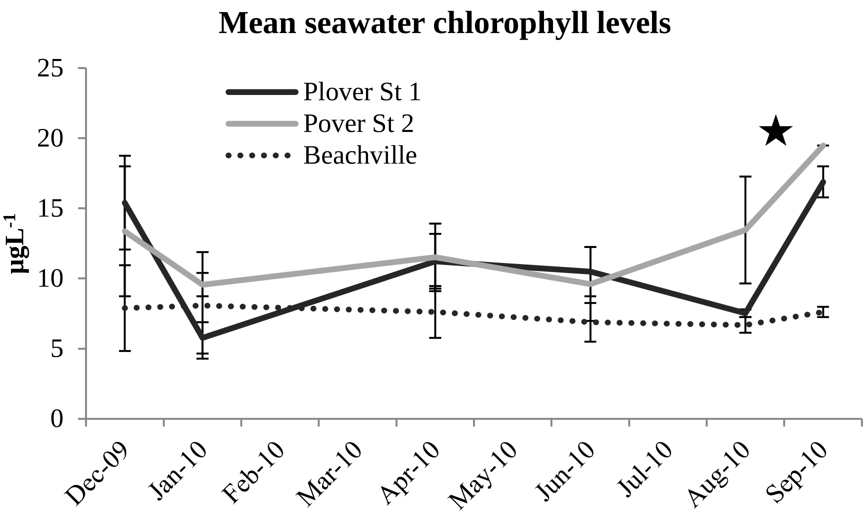


Figure 5.14: Mean seawater chlorophyll levels (± 1SE) from the Plover Street transplant and Beachville Road control sites for the trial period, December 2009- October 2010. ★ September 4th 2010 earthquake.

Mean seawater chlorophyll levels showed significant differences between both site and sampling time (Table 5.5) with values falling between 5 and 20 μgL^{-1} . The Beachville Road control site chlorphyll levels showed the least variation over the time of the trial, but there were definite fluctuations at the two transplant sites (Figure 5.14).

Table 5.5: Results of 2 way ANOVAs without replication comparing seawater chlorophyll levels, TVS and pore water content at the 2 Plover Street transplant sites and the Beachville Rd control site for each monitoring period. Significant values are shown in bold.

Factor	**Source**	***df***	***F value***	***P-value***
Pore water	Site	9	2.15	**0.041**
	Sampling time	6	10.22	**0.002**
TVS	Site	9	1.46	0.187
	Sampling time	6	2.96	**0.014**
Chlorophyll	Site	5	3.54	**0.004**
	Sampling time	5	4.67	**0.015**

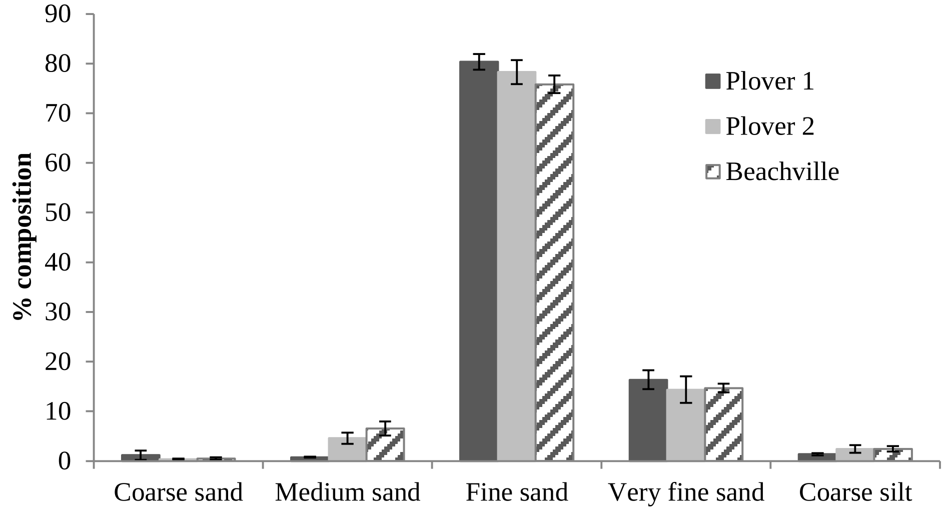


Figure 5.15 : Mean percentage composition (± 1 SE) of the sediments at the transplant and control sites.

The sediment at all three sites was predominantly fine sand (ANOVA p >0.99) determined from samples collected at each site monitoring exercise (Figure 5.15).

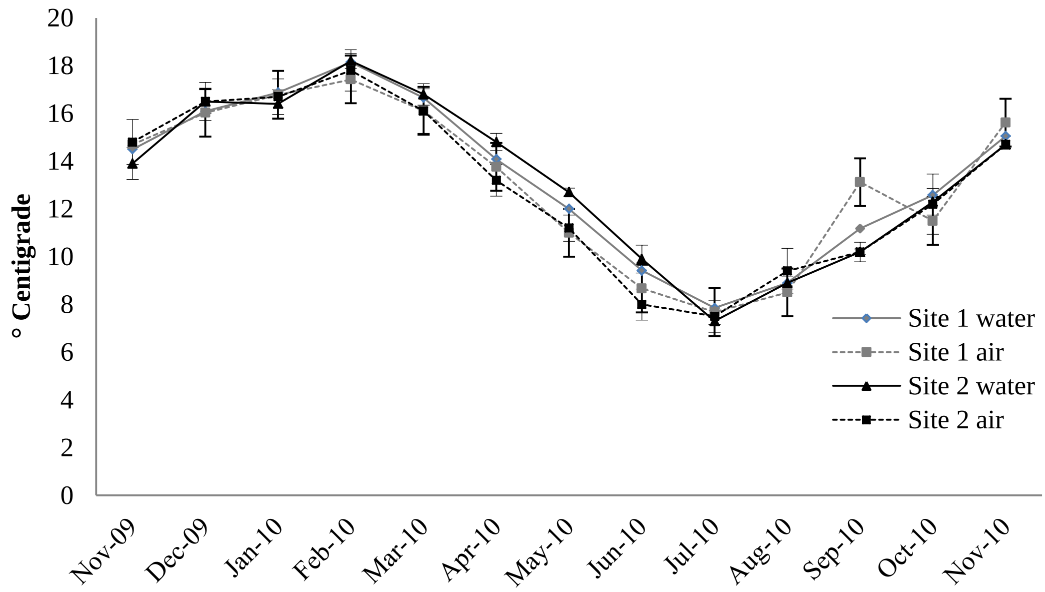


Figure 5.16: Mean monthly water (high tide) and air (low tide) tempertatures at the two transplant sites at Plover Street. Error bars are SE.

Table 5:6: Result of 2 way ANOVA describing differences in mean monthly air and water temperatures at the Plover Street transplant sites.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Site	1.15	3	0.38	1.05	0.382
Time	537.61	12	44.8	122.5	**0.001**
Error	13.17	36	0.37		
Total	551.93	51			

Mean monthly air and water temperatures (Figure 5.16) which ranged between 6 and 18°C did not show significant differences between sites, but there were significant differences for both with time (Table 5.6). Maximum temperatures during summer were in the vicinity of 18°C (Figure 5.16). Winter (2010) temperatures were at their lowest in July (7°C).

Condition indices for the transplant treatments which ranged between 120 and 150 (Figure 5.17) showed significant differences (p = 0.001) between sites (Table 5.7). At the time of the final lift (November 2010) cockles from Plover Street site 1 (east) had lower CI gravimetric values (120) than both Plover Street 2 (west) (145) and Beachville Road (150). Naturally occurring cockles gathered from within the uncaged plots and from outside the transplant areas showed significant CI differences with Plover Street site 1 being the poorest (130), and site 2 (150) being better than Beachville Road (135). However, with the uncaged transplanted cockles there was little difference in CI values between Plover Street site 2 and Beachville Road (140-142). There were significant differences in CI between the various treatments (p= 0.019) (Table 5.7) within the three sites. At the two transplant sites at Plover Street a similar pattern emerged with naturally occurring cockles within the sites and within the 300mm x 300mm plots within the sites being in better condition than both caged and uncaged transplanted ones. Naturally occurring cockles gathered from outside the transplant sites were in even poorer condition.

At the Beachville Road control site, the transplanted cockles were in better condition (CI 140) than the two categories of naturally occurring cockles (130-135).

Table 5.7: Repeated measures ANOVA describing differences in mean gravimetric condition index for caged, uncaged and naturally occurring cockles at the Plover Street transplant sites and the Beachville road control site at the final lift, October 2010. Significant values are in bold.

Source of variation	*df*	*MS*	*F*	*p-value*
Site	2	3742	10	**0.001**
Treatment	5	1073	3	**0.019**

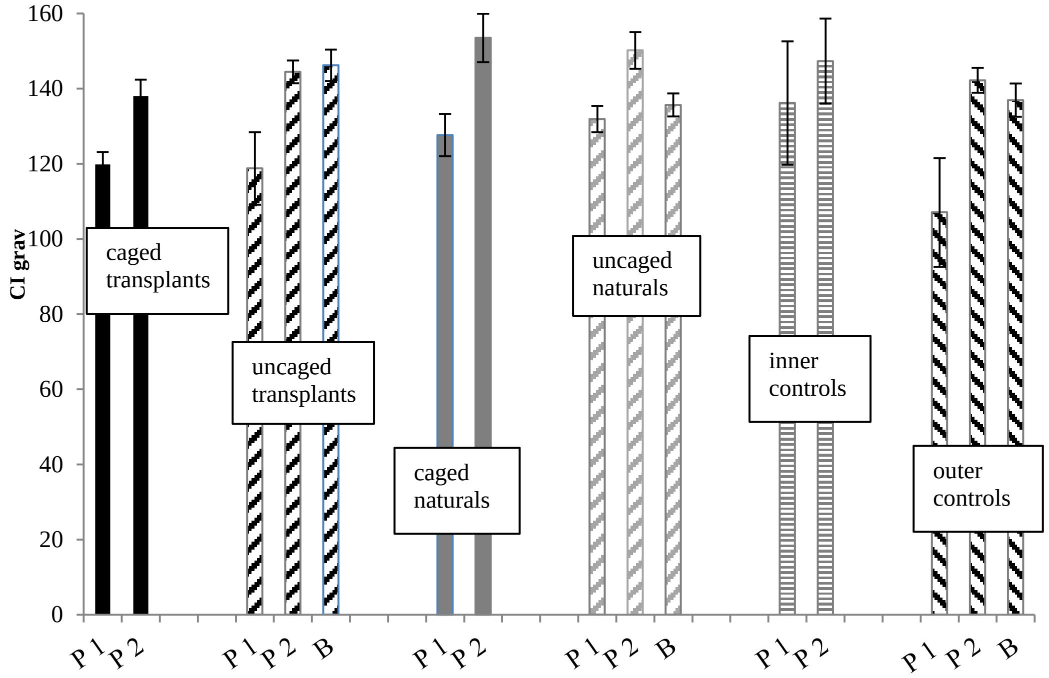


Figure 5.17 Plover St transplant experiment. Mean gravimetric condition index (±1SE) of caged and un-caged, transplanted and naturally occurring control *Austrovenus stutchburyi* collected from two sites at Plover Street (P1 and P2) and Beachville Road (B) at the end of the experiment, October 2010. Also shown are the values for controls outside of the experimental plots.

There were no significant differences (Table 5.8) in gravimetric condition index between transplanted (CI range 105-155) and natural controls (CI range 104-172) at the Beachville Road control site at each of the sampling times. Caged (CI range 120-138) and uncaged (CI range 118-145) transplanted cockles also showed no significant difference in gravimetric condition index (Figure 5.17). Natural cockles showed slightly higher condition indices than those treated to the transplant regime. However, there were significant differences (p = 0.001) in CI for both treatments over time with a slight drop over autumn and an increase at spring (Figure 5.18).

Table 5.8: Repeated measures ANOVA describing gravimetric condition index for transplanted and control cockles collected each sampling time from Beachville Road for the open cage experiment October 2009 –September 2010.

Source of variance	***df***	***MS***	***F***	***p***
Time	5	482	6	**0.001**
Treatment	1	201	2	0.209

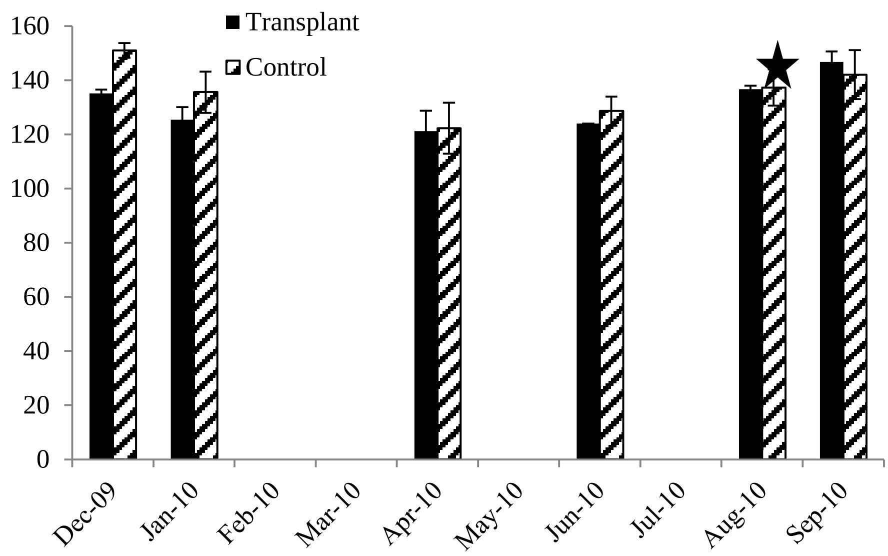


Figure 5.18: Mean condition index (gravimetric) (±1SE) of transplanted and natural cockles collected from the control site at Beachville Road for the open cage experiment.
★ **September 4th 2010 earthquake.**

Mitochondrial Cytochrome c (CO1)

No unique haplotypes were identified when subsamples of cockles from Otago and Canterbury were compared for the mitochondrial CO1 gene. The analysis identified four common haplotypes (A,B,D, and F,) suggesting that these cockles may belong to the same subpopulation (Ross 2011) (Figure 5.19).

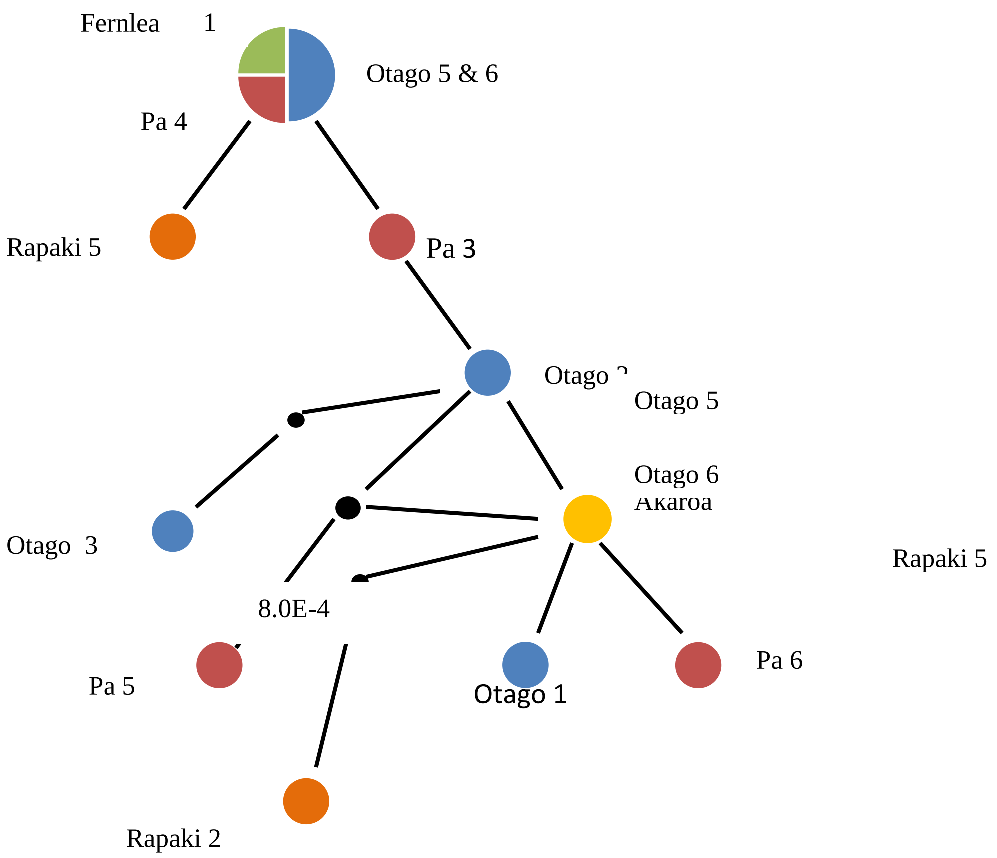


Figure 5.19: Cladogram describing connectivity of *Austrovenus stutchburyi* from 4 sites.

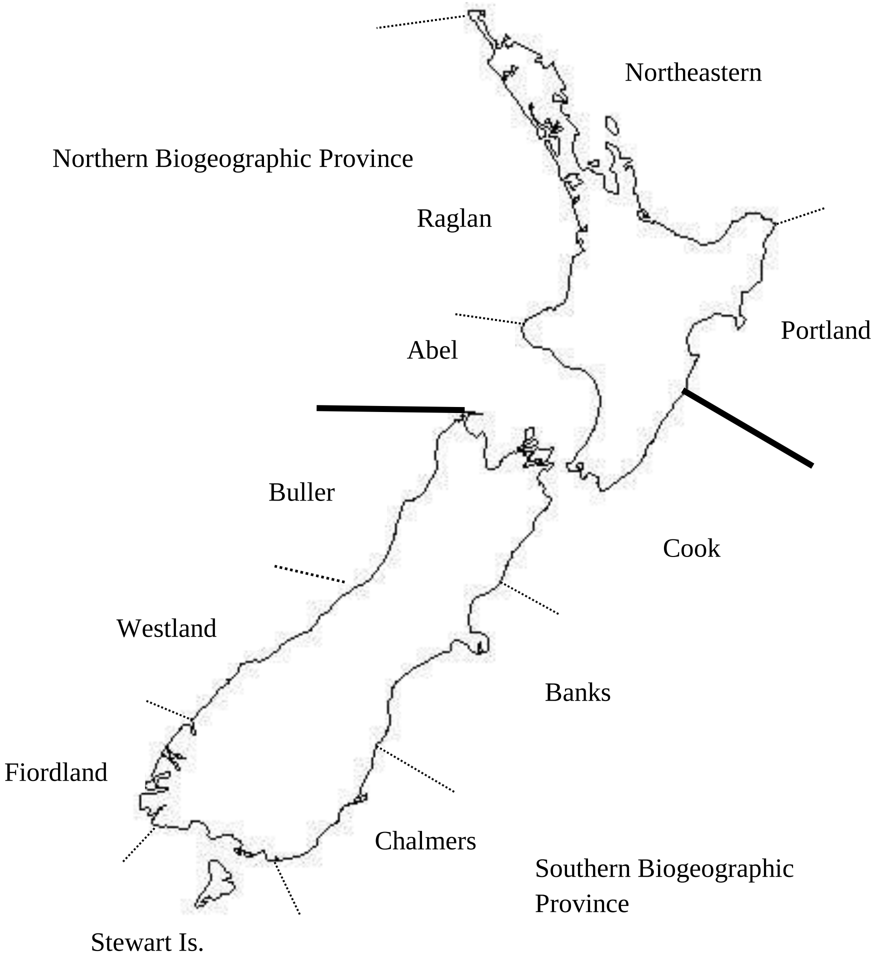


Figure 5.20: Coastal biogeographic provinces and regions of New Zealand. Based on map by Ross (2011)

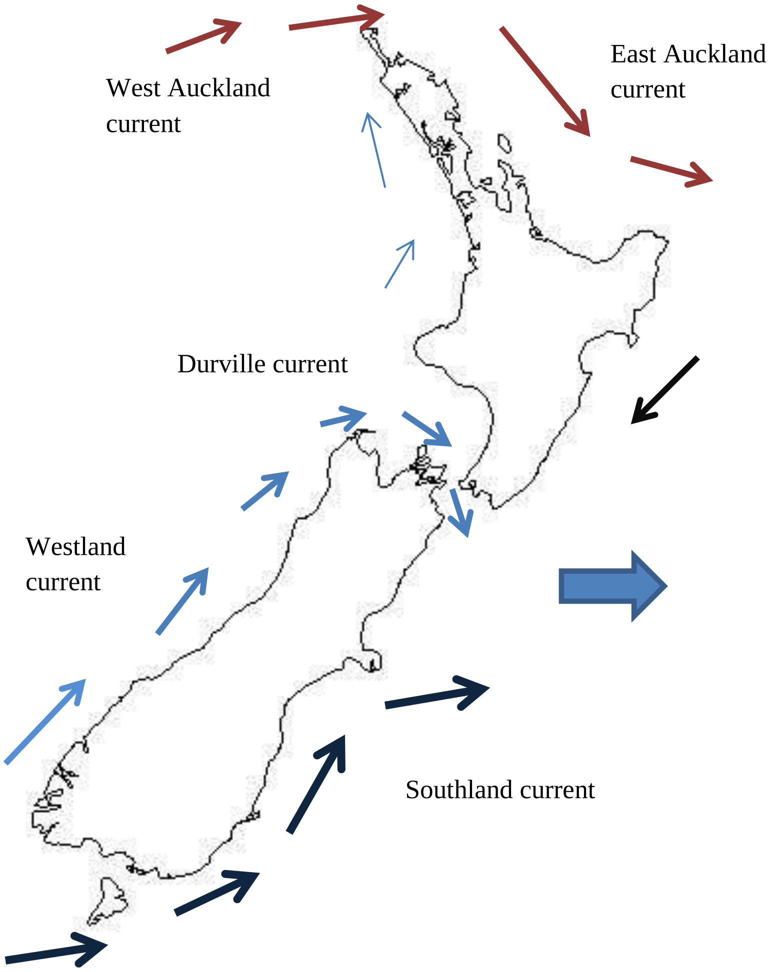


Figure 5.21: Map of New Zealand showing main ocean currents.

Discussion

The variety of experiments carried out in this research using *Austrovenus stutchburyi* as the test organism allowed the assessment of several options for transplanting this particular bivalve. These experiments highlight that a number of factors e.g. location, sediment and salinity are likely to have an effect on the success of the transplant. Transfer of adult bivalves is a feasible option, simpler to carry out than raising and seeding spat (Stewart & Creese 2002; Gosling 2003).

The preliminary twelve week experiment between estuaries used three shell length class ranges of New Zealand cockle transferred from Beachville Road to three of the Canterbury estuaries used previously in the population (Chapter 3) and condition (Chapter 4) research, namely Saltwater Creek, Avon-Heathcote/Ihutai and Akaroa Harbour. From an environmental perspective these results were interesting - survival of transplanted cockles was lower at the high salinity Tern Street site in the Avon-Heathcote/Ihutai estuary with no class size being any more successful than another, while site sourced cockles (the control) had more small cockles surviving. This suggests that even though salinity levels affect growth and health, they may not be the sole controlling factor in cockle survival, and that *A.stutchburyi* is relatively tolerant of a range of salinities (Marsden 2004; Tallis 2004). At the low salinity Takamatua site, survival rates for both transplanted and control cockles were high across all class sizes even though the natural population is comprised of small to medium sized cockles. This was a short term experiment, and the transplanted cockles were confined in cages at low densities (30cockles/cage) compared with the density of naturally occurring cockles at this site (>100 cockles/25cm x 25cm quadrat). Un-caged transplanted cockles or higher densities may have resulted in a reduction in condition due to completion for food and space. At all three transplant sites, the caged site specific control cockles survived better and were in better condition than their transplanted counterparts. This raises the question of stress effects from handling, as the control cockles were gathered at the time of the transplant and not subjected to any travel or time in an aquarium as were the transplanted ones. Research to date has not identified any adverse effects of transportation to handling (Cummings et al. 2007) with *A.stutchburyi* being described as a resilient species. The current research suggests that transportation effects on cockles should be further investigated.

Following on from this preliminary experiment, a longer term (6 month) trial was established taking cockles from Plover Street and placing them at the Bromley waste water outlet, and back close to their source at Plover Street. This exercise was terminated after 12 weeks due to poor survival rates at both sites. Live cockles collected at the two sampling opportunities did not show site differences in condition indices even though the two sites have different environmental attributes such salinity and sediment structure with Plover Street having salinity levels about 32ppt and a fine sand substrate. Bromley, a potentially more contaminated site, had a more silty substrate, with salinity levels of 30ppt (see Chapter 3). The sediment at Bromley is usually anoxic. The experiments failed because in the fully enclosed cages, the cockles were not able to move vertically in the substrate and died either of smothering through sediment build-up, or exposure as a result of severe scouring around the cages.

The long term large scale open cage transplant followed the design used by Cummings et al. (2007) with a density of 50 cockles per 30cm x30cm plot as they recommended.

In this experiment, cockle survival rates varied between sites and between treatments with caged cockles having a higher survival rate than un-caged ones. This latter finding is probably attributable to the non-retrieval of uncaged cockles. Site one (east) was better than site 2 (west), with the un-caged cockles surviving at higher numbers than the caged ones at site 2. However, the trend was increasing mortality over time, with approximately 45% of the total (caged and uncaged) transplanted cockles being alive at the end of the twelve month trial which was higher than that experienced by Cummings et al. (2007) in their experiment which ran for a similar time span. Their extensive transplant study was carried out in Whangarei Harbour, using adult cockles 25-30mm shell length. The sediment at this site was >90% fine sand and two densities of cockles were assessed (20 and 75 individuals).

Approximately 30% of the transplants remained after 1 year with mortality being associated with weather conditions (wind, temperature) and the stress of being caged at higher densities. In the present study the effects of wind and extremes of temperature were negligible, and the cockle density was lower (50/30cm x 30cm cage) compared with the high density (75/30cm x 30cm cage) used by Cummings et al. (2007).

A pre-transplant census at both sites showed *Austrovenus stutchburyi* as the dominant species followed by *Paphies australis* and *Diloma subrostrata.* These three species again dominated the post-transplant census with significant increases in density.

Cockles from Plover Street site one (east) had lower condition indices (but higher survival) than the other sites. At both test sites, the un-caged naturally occurring cockles were in better condition than any of the transplanted ones. At the Beachville Road control site condition was assessed each sampling time, with no significant differences between transplanted and natural cockles, although there was a difference in condition associated with time (seasonal). This result suggests that while caging may give protection from predation, these gains in survival may be offset by a loss of condition attributed to competition effects brought about by the restrictive nature of the cages. Overall, this long term experiment was successful in establishing the viability of adult cockle transfer with reasonable survival rates and levels of condition.

Levels for some the environmental factors assessed in this long-term study were similar for both test sites and the control site (sediment, total volatile solids (TVS) and air and water temperatures) with site differences for pore water content and chlorophyll levels. While these differences were obvious before the earthquake, there was a marked increase in chlorophyll at both Plover Street sites after this event and a decrease in pore water. There was also a marked increase after the earthquake in TVS at Plover Street site 2 and to a lesser extent at Beachville Road (Zeldis et al. 2011). These changes may be explained by changes to the estuary in general and by disruption to the waste water services for Christchurch city. The water flow in the estuary has been altered through an up-lifting of the estuary bed at its mouth and a corresponding drop at the mouth of the Avon River flowing into the estuary. None of the test sites was affected by liquefaction within their boundaries, although it was apparent around much of the area.

With ever increasing human populations making demands on the world's resources, the concept and practise of 'stewardship' remains vital to ensure the continuance of species' existence through the sustainable use of all resources. Human activities are responsible for habitat degradation especially through land development and overharvesting. Habitat changes through land development can result in sedimentation, nutrient loss, altered water flows, and introduction of pollutants such as heat and chemicals. Harvesting often results in the removal of essential breeding stock, the larger older individuals that provide the gametes which will produce the on-going generations.

To offset the damage and changes to natural systems there are conservation efforts to reinstate and sustain ecosystems including harvesting bans, and transplants. Previous research (Morrison & Browne 1999; Voller 2006) has established that shellfish bed closures by themselves may not be sufficient to increase shellfish production due often to irregular recruitment. With decreasing bivalve populations, restoration or supplementation processes are becoming necessary to ensure the continuation of these areas.

From this thesis research, and from other trials carried out elsewhere (Dobbinson et al. 1989; De Luca-Abbott 2001; Stewart and Creese 2002; Stewart 2005; Norkko et al. 2006; Cummings et al. 2007) a number of factors influencing the success of bivalve transplantation have emerged, confirming that while transplanted cockles do survive this can be poor in the most contaminated sites. Small-scale transplants of cockles have investigated survival and growth with tidal height being a major factor affecting clam growth rate (Dobbinson et al.1989) with the longer immersion period for low-tide bivalves, together with the associated higher food availability explaining the high growth rates in clam populations exposed to normal salinity conditions. Stewart and Creese (2002) also found that cockle growth rate was faster lower in the shore. Other experimental transplants undertaken as part of biomarker studies assessing the survival and health of cockles transplanted from clean sites to those exposed to contaminants or increased sedimentation (De Luca-Abbott et al. 2000; Stewart 2005; Norkko et al. 2006) also identify the importance of substrate characteristics in bivalve survival.

As part of their traditional management of kai moana (sea food), Maori have specific practices to protect and maintain the resource. Much of the knowledge exists as oral tradition and there are no detailed written descriptions of predator removal or shellfish transfer (Booth & Cox 2003). Concern about the natural environment, including the state of shellfish beds, by various parties including Maori, commercial and recreational fishers, has identified a need to develop techniques that optimise restoration success. Projects involving scientists, community groups and commercial interests directed towards whole ecosystem restoration and management as a means to boosting bivalve populations have been trialled in the USA (Rice et al. 2000).

Some of the potential threats to cockle beds in New Zealand, both in their natural state and under remediation, include natural events such as earthquakes, tsunami, and storms (Stewart and Creese 2002) and factors of anthropogenic origin. Sedimentation (from development and agriculture) could cover beds to depths to such an extent that cockles cannot unbury themselves. Contaminants and reduced salinities can impact on cockle growth and limit the maximal size of shellfish. Predators, including eagle rays (Hines et al 1997), oyster catchers, whelks and crabs that nip off the exposed cockle siphons may also influence survival. However, some caging studies (Cummings et al. 2007; this thesis) suggest that predators may not be as great a threat with a large cockle with a thick shell having a size refuge from predation.

In New Zealand, urban development is often around estuaries putting these areas under considerable pressure from contaminants and anthropogenic changes. The positive note is that these modified habitats are likely to be the prime sites for future restoration as the New Zealand Resource Management Act (1991) requires local councils to minimise the effects of sewage and water discharges into the ocean. As a result of these regulations the Christchurch City Council is an example of a municipality that has constructed an off shore pipeline, and today cockle beds are better established in some parts of the estuary than they were some 50 years ago (Marsden & Adkins 2010).

Commercial enhancement of intertidal bivalve beds is generally carried out through two techniques. The first option is to spawn and rear spat in a hatchery and/or collect it from the wild. The second is to relocate spawner transplants from an area where the species is abundant to areas where the stocks have been depleted as has been used for the hard clam *Mercenaria mercenaria* in the United States (Doall et al. 2008).

In other parts of the world spat is produced for use in reseeding shellfish beds (Sturmer et al.2003) and while the production of spat is possible for *A.stutchburyi* (Stephenson and Chanley 1979; Stewart & Creese 2002) it has not been done on a commercial scale. The New Zealand cockle spawns over prolonged and variable timeframes (Stewart 2005; this thesis) with a pelagic larval stage of 20 days at an average temperature of 25^{o}C (Stephenson & Chanley 1979; Ross 2011) which should allow widespread dispersal based on hydrography and currents (Lindquist et al 2004; Lindquist et al 2009) although research to date suggests that there are biogeographic barriers that limit the dispersal of larvae (Ross et al. 2009; Ross 2011).

On a non-commercial basis, a more economically viable option for bed enhancement is the transfer of mature adults with numerous experimental studies investigating growth and survival in transplanted bivalves (Dobbinson et al 1989; Thrush et al 1996; De- Luca Abbott 2001; Norkko 2005; Stewart 2005; Cummings et al. 2007). Although transplanted cockles survive, their ability to grow, reproduce and establish new populations requires further investigation.

Timing of transplant exercises may be important. Peterson et al. (1995) found survival of hard clams in USA was higher in the autumn and winter than in summer, and similar survival differences were identified for New Zealand cockles by Cummings et al. (2007). While this thesis research and that of Cummings et al. (2007) has shown that larger clams have good rates of survival, and transplants can be done at any time of the year in areas with adequate nutrient levels, transplants are most likely to be successful in early spring when the bivalves are most active, and somatic and shell growth is highest.

Caged transplanted cockles have the potential to be protected from predation, and survival rates of these were higher than those of un-caged transplants. But, the survival rates established in this research may not be an accurate picture of the reality of what was happening. It is accepted that clams are mobile and move through and across the substrate. The unaccounted-for cockles from the un-caged plots may not all have been victims of predation, but may have travelled beyond the outer boundaries of the study sites. Caging cockles can have negative impacts on condition and fully enclosed cages can result in increased mortality by restriction of vertical and horizontal movement through the substrate. While caging affords a degree of protection from predation and aids monitoring exercises, caged cockles had poorer condition than un-caged ones.

The non-detection of unique haplotypes in the analysis of mitochondrial cytochrome *c* subunit 1 suggests good connectivity between the source sites of the cockles tested, and supports Ross's (2011) findings of no significant differences in populations in the SE region (lower east coast, South Island) of New Zealand. He identified differences between the two main islands, and establish that the genetic boundaries corresponded partly with the biogeographic breakpoints defined by Shears et al. (2008). Based on these findings, there should be no issues with transferring cockles between the South Island's Chalmers and Banks coastal bioregions (Figure 5.20), especially if the movement is limited to a south to north direction, the direction of the prevailing water currents (Figure 5.21) which would direct natural larval dispersal.

Based on these findings, a number of recommendations are proposed to improve the population dynamics of benthic bivalves by way of transplanting mature adults:

a. Evaluation of the potential restoration site to identify physical (e.g. sediment structure, salinity), chemical (contaminants, oxygen levels) and biological characteristics (benthic organisms and nutrients) and any possible risks (sedimentation, predators, toxic blooms). Sites do not need to be uncontaminated as long as other stressors are excluded or their potential effects recognised, e.g. interactions between high nutrient and low oxygen levels.

b. Large scale, un-caged transplants into the mid-to low-tide shore level, of mature adults (25-30mm shell length) preferably in areas known to support cockle populations.

c. Regular monitoring, both for survival and condition of cockles, especially after extreme weather events. Also monitoring for recruitment.

d. On-going consultation with local community and other stakeholders.

To summarise, un-caged, high volume, larger sized, transplants into clean sandy areas in mid to low tide regions with high salinity and good nutrient supply should ensure long term survival of well-conditioned cockles with the potential to reproduce and restock the area. Involvement of locals and stakeholders will aid monitoring for interference, and after extreme environmental events.

Finally, it is necessary to establish criteria to determine if the restoration process is successful. There will be different views ranging from sufficient densities to support the sustainable harvesting of the shellfish through to including the role of bivalves in the maintenance and production of benthic habitats and their importance in maintaining species diversity. As part of the cultural and spiritual heritage of New Zealand the restoring of shellfish beds should be a priority in the management of our coastal ecosystems.

Chapter 6

Summary and General discussion

All over the world declining shellfish populations have been recorded (Airoldi & Beck 2007; Polyakov et al. 2007; Genelt-Yanovskiy et al. 2010; Vassiliev et al. 2010; Watanabe & Katayama 2010; Yan et al. 2010; Ravit et al. 2012), affecting natural and commercial beds alike. Factors including human exploitation (Atkinson et al. 2010), temperature, salinity and food supply (Hofman et al. 2006; Leontarakis et al 2008; Guerra et al.2012) and sediment quality and dynamics (Bouma et al. 2001; Huxham & Richards 2003) have been identified as influencing bivalve population structure and sustainability. Growth rates, abundance and survival of bivalves are affected by sediment structure (Hermann et al. 2009; Lundquist et al. 2009) with coarse grain sizes inhibiting burrowing. Stewart (2005) noted that, in New Zealand, many of the estuaries that support *Austrovenus stutchburyi* populations are close to the highest densities of human population with the associated potential for urban development impacts, and increased harvesting pressure. My thesis research investigated the population structure and dynamics of eight cockle beds in four estuaries in the Canterbury region of New Zealand from December 2006 through to December/January 2012 to identify the factors that may limit the establishment and growth of this species. Condition and reproductive potential were also assessed in parallel with the population census. A series of transplant experiments investigated the potential of using mature cockles as a cost effective, practical way to to supplement beds where populations are not as well-stocked as they were historically. A number of key questions were raised which can now be answered.

The first question was: Does the population structure vary between cockle beds in the Canterbury region? The present research affirms that at several sites in the Canterbury region cockle beds are similar to beds elsewhere in New Zealand and other parts of the world, in that population density, length classes, condition, growth and reproductive potential are variable both within the areas i.e. between the paired sites, and between the areas. Recruitment is sporadic, both between sites and temporally- as has been found for other species. This leads to question 2: Do environmental conditions affect the health, growth and reproduction of shellfish? i.e. does cockle condition correlate with environmental factors? Cockle length and size were positively correlated with water temperature, but not with other environmental factors assessed, in particular salinity, and sediment structure. However, there was a positive significant correlation between mean condition index and salinity. This research has confirmed that environmental factors do have an effect on cockle growth, and condition.

Question 3 asked: Are cockle beds in the Canterbury region in decline? Although some of the beds do not support dense populations, the numbers remained constant over the seven years of the research, and there was evidence of recruitment. In the main, therefore, the Canterbury beds cannot be described as in decline. However, the site adjacent to the marae at Port Levy/Koukourārata is cause for concern. Harvesting has been banned there, followed by severe collection restrictions, for over a decade, and while the numbers are constant, they are very low. Sedimentation may be a major factor here, with additional material making its way into this inlet from Lyttleton Harbour. It was thought that the mussel farms at the seaward end of the inlet could impact on the amount and quality of food available for other species of filter feeders , but the microorganic levels at this area did not differ from those at the other sites.

Question 4 addressed whether shellfish beds can be successfully enhanced by habitat restoration or improvement? An affirmative answer to this is illustrated by the increase in numbers of all species into the trial beds. Disturbance of the area, along with increased pelagic-benthic coupling occurring associated with the increased numbers of water filtering cockles, may result in an environment more suitable for these other species. This cannot be described as restoration but rather rejuvenation, as we cannot take an area back to its original pristine condition but can control/limit anthropogenic impacts which are having a negative effect on population dynamics.

Cockle transplants can be used to improve or maintain the long term sustainability of shellfish beds. This ties in with question 4. Cockle transplants not only add to the benthic biomass, the cockles are also playing a large role in the cycling of nutrients in the ecosystem and by filtering water reduce turbidity, allowing the growth of sea grasses, which in turn modify the environment making it more suitable for other species to colonise.

Not only are questions answered in this thesis, several others are raised. What is the biggest threat to cockle survival? Humans and the pollution, habitat disruption and harvesting that they instigate. With overharvesting, we have to question the wisdom of a 150 cockle/day/person bag limit with no size restriction for most of the beds in New Zealand. The Auckland /Coromandel region has a limit of 50/ day and there are other limits in reserves (e.g.Rapaki). From personal experience, I know these limits are not adhered to, with the excuse often being that the restrictions are not known, the signs not seen or are not in a language which is understood. Historically, stewardship techniques were practised such as taking only sufficient cockles for a meal, and within settlements, people respecting each others harvest areas.

Another question to be addressed is what are the impacts on the donor bed is going to be by taking adult cockles for transplantation elsewhere. As stated earlier, cockles are not only a food source for humans, they supply nutrients for other species, and are a vital part of the physical structure of the ecosystem , involved in pelagic-benthic coupling and providing habitat for other species Kainamu 2010).

The population characteristics established from this research showed that not all the study sites would be suitable sources for cockles for transplanting, with the main defining characteristic being the density of adult (25-30mm length) cockles. It would be counterproductive to remove adults from a lower density site. An area for further research is transplanting the small cockles from Takamatua at varying densities into a known healthy site such as the Plover Street area of the Avon-Heathcote/Ihutua Estuary and monitoring growth and condition. In parallel with this, cockles from Plover Street could be confined in cages at Takamatua at higher densities and for a longer length of time than was used in the preliminary transplant exercise between estuaries. From this it should be possible to identify the role of intraspecific competition (for nutrients) on the growth and survival of cockles.

Areas for cockle (*A. stutchburyi*) transplantation need to suit their habitat preferences with regard to substrate composition, salinity and nutrient availability. This bivalve occurs on estuarine sand flats with freshwater inputs, with grain sizes ranging from fine sand to slightly silty. Anoxic sediments can result in cockle mortalities and therefore it is important to consider the history of previous anoxic events. Cockles are relatively tolerant of salinity fluctuation, but continued low salinity levels have been correlated with reduced growth and condition (Marsden 2004). Best cockle growth would be expected to occur in high salinity sites. While some trace elements are essential for metabolism, other have the potential to accumulate in toxic levels (Rainbow 2002). Transplant sites need to be selected relative to other species to reduce competition and predation. The present study and other research suggest that cockles would survive in a wide range of trace metal concentrations provided there were few other stressors. In cockle transplants consideration has to be given to the presence of other species. Cockles and pipis can occur adjacent to each other on the shoreline, but not to any extent in mixed beds. Placement of the cockles need to minimise disruption to the pip beds. Introduction of cockle into seagrass beds could have mixed benefits. On the positive side there is an increase in nutrient availability and sheltered larval habitat. The downside is that sea grass offers the same benefits to cockle predators- whelks and crabs (Linquist et al. 2009).

Other New Zealand bivalve species which are showing population declines and are potential transplant candidates include members of the genus *Paphies* which occur on ocean beaches and sandy banks of estuaries where there is good water flow. *P. australis* the pipi, found in both the North and South Islands is not tolerant of reduced salinities (Marsden & Knox 2008). The tuatua (*P. donacina*) occurs in the low tide high energy region of ocean beaches rather than in estuaries where it probably contributes to the bed stability (Ministry of fisheries summary 2009). There are more tuatua beds in the North Island than the South Island and since its inclusion in the Quota Management System there has been restricted commercial harvesting of this species with most of the take being recreational. Adult *P. ventricosa* (toheroa) favour the mid-tide region of fine sand beaches with larval recruitment occurring in the high tide region. As a result of over exploitation this species is protected under a harvesting ban with the only legal harvest at Oreti Beach in Southland. Māori customary take is authorised by kaitiake representing the appropriate runanga (Beentjes 2010). While these species have different habitat requirements to *A.stutchburyi,* all have historical significance for Māori as kai moana, and as such their husbandry needs protecting and restoring.

As a result of this thesis research some guidelines and protocols can be established for transplanting bivalves:

1. The habitat has to suit the species being transplanted with regard to salinity, sediment, temperature.

2. Shellfish should not be transferred to areas where there are high levels of contaminants and/or anoxic conditions.
3. The transplant should not interfere with other natural communities.
4. Shellfish for transplant need to be free from disease and toxic algae.
5. Should be transferred within the natural connectivity distance of prevailing water currents (Figure 5.21).
6. Prior to any large scale transplants preliminary trials should be carried out. It might be appropriate to use cages to facilitate monitoring of survival and condition.
7. Community involvement should be encouraged at all stages of the transplant.
8. Restorations need to be well-designed and transplanted shellfish monitored regularly.

The future for bivalve transplants in New Zealand looks positive. Research shows that survival and condition can be maintained in transplanted individuals. If transplants are successful then it is expected the shellfish will go on to reproduce allowing natural recruitment to supplement the restoration exercise. Science, local knowledge (Māori oral history and customs) and communities working together will see positive changes not only at the population level, but to the environment in general. This will ensure that shellfish resources are enhanced for future generations.

Ko ngā awa ngā moāna me ngā whenua te wai ū mō ngā uri whakatipu.

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Appendices

1. Species list
2. Allozyme sequences

Appendix 1;

Final lift species count: Treatment 1: caged; 2 uncaged; 3 inner control; 4. Outer control;

Site	Treatment	Quadrat	*Austrovenus stutchburyi(red)*	*Austrovenus stutchburyi (nat)*	*Paphies australis*	*Macomona liliana*	*Xenostobus pulex*	*Diloma subrostrata*	*Amphibola*	*Cominella galndiformis*	*Cellana radians*	*Amaurochiton glaucus*	*Halicarcinus whitei*	*Helice crassa*	*Macrophthalmus hirtipes*	*Anthopleura aureoradiata*	*Polychaete green*	*Polychaete red*	*dead red*
1	1	1	44	85															
1	2	2	16	69															
1	1	3	50	185															
1	2	4	4	138															
1	2	5	19	70															
1	1	6	50	113															
1	2	7	0	210															
1	1	8	42	99															
1	1	9	28	125															
1	2	10	3	159				17	1	4									
1	1	11	28	94															
1	2	12	1	25															
1	2	13	9	29															
1	1	14	27	16															
1	2	15	11	49															

1	1	16	9	16										
1	3	*1*	0	207	1		5	1						
1	3	2	0	77	2		15	5						
1	3	3	0	48	4		4	2						
1	3	4	0	50	2		4						1	
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1	4	3	0	84	1		2	1	1					
1	4	4	0	18	11		7	1						
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2	2	7	4	22			2				1			
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2	1	9	35	84	16		17	2						
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2	2	12	38	28	2	1	2							2

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2	2	15	11	49											2
2	1	16	9	35											5
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3	7	3	0	60	4		8	6	1	1					
3	7	4	0	56	9		16	1	3						
3	6	1	2	101	26		72	4				1		1	
3	6	2	0	86	20		14	1			1			1	1
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3	6	4	3	83	25		4	1			1				
3	6	5	0	88	19		13	2	1		1				

3	6	6	3	64	18	8	40	2		1	3
3	6	7	0	52	3	5	2				
3	6	8	0	82	11	34	2	1			
3	6	9	1	59	16	7	4		1		1

Appendix 2.

>Akaroa_specimen

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